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(54) Title: FLOWERING TIME MODIFICATION

(57) Abstract: Recombinant polynucleotides and methods for modifying the flowering time of a plant are provided. Plants transformed with the recombinant polynucleotides may have flowering times that are accelerated, delayed or induced under specific conditions. Additionally, transformed plants may have altered vernalization requirements.

## FLOWERING TIME MODIFICATION

The present invention claims priority in part from US Provisional Application Serial Nos. 60/159,464 filed October 12, 1999; 60/164,132 filed November 8, 1999; 60/166,228 filed November 17, 1999; 60/197,899 filed April 17, 2000; and Plant Trait Modification III, filed August 22, 2000.

### FIELD OF THE INVENTION

This invention is in the field of plant molecular biology and relates to compositions and methods for modifying a plant's flowering time or vernalization requirements.

### BACKGROUND OF THE INVENTION

In order to maximize reproductive success, plants have evolved complex mechanisms to ensure that flowering occurs under favorable conditions. Analysis of late flowering mutants and ecotypes in *Arabidopsis* has revealed that such mechanisms are based upon several genetic pathways which may contain 80 or more genes (Martinez-Zapater and Somerville, (1990) *Plant Physiol.* 92:770-776; Koornneef et al. (1991) *Mol. Gen. Genet.* 229:57-66; EM Meyerowitz and CR Somerville Eds (1994) *Arabidopsis* pp 403-433 Cold Spring Harbor Laboratory Press, New York). Together these loci co-ordinate flowering time with environmental variables (e.g. day-length, temperature, light quality, and nutrient availability) and with the developmental stage of the plant.

*Arabidopsis* flowers rapidly when grown under long day conditions of 16 hours or continuous light, but flowers much later under short day conditions of 8 or 10 hours light. Genes regulating this response constitute the photoperiod pathway and were identified by mutations that cause late flowering under long day conditions but which do not alter flowering in short day conditions. Examples from this group, which promote flowering in response to long days, include *CONSTANS* (CO), *GIGANTEA* (GI), *FT*, *FWA*, *FE*, *FD*, and *FHA*. A second group of genes, which includes *LUMINIDEPENDENS* (LD), *FCA*, *FVE*, *FY*, and *FPA*, form an autonomous pathway that is active under all day-length conditions. Mutants for this second class of genes flower later than wild type controls irrespective of the day length conditions (Koornneef et al. (1991) *Mol. Gen. Genet.* 229:57-66; EM Meyerowitz and CR Somerville Eds (1994) *Arabidopsis* pp 403-433 Cold Spring Harbor Laboratory Press, New York).

In addition to differing in their response to day-length, mutants from the photoperiod and autonomous pathways show a differential response to prolonged cold (vernalization) treatments (Vince-Prue, (1975) *Vernalization*. In *Photoperiodism in Plants* pp 263-291, McGraw Hill, London) Through a vernalization response, *Arabidopsis* ecotypes from Northern

latitudes, such as Stockholm, are adapted to flower in the spring following exposure to cold winter conditions. This avoids flowering in the late summer when seed maturation might be curtailed by the onset of winter conditions (Reeves and Coupland, (2000) *Curr. Opin. Plant Biol* 3:37-42). When these ecotypes are grown in the laboratory they flower late, but will flower  
5 much earlier if subjected to a cold period of 4-6 weeks during seed germination. In a comparable manner, mutants from the autonomous pathway exhibit a very marked reduction in flowering time when subjected to vernalization. In contrast, mutants from the photoperiod pathway only show a minor response to cold treatments (Chandler *et al.*, (1996) *Plant J.* 10:637-644; Koornneef *et al.*, (1998) *Genetics* 148:885-892). Thus, vernalization can  
10 overcome the requirement for the autonomous pathway conditions (Reeves and Coupland, (2000) *Curr. Opin. Plant Biol* 3:37-42).

Two *Arabidopsis* genes, *FLOWERING LOCUS C*, *FLC* (also known as *FLOWERING LOCUS F*, *FLF*) and *FRIGIDA* (*FRI*), act in conjunction to repress flowering in the absence of a vernalization treatment (Napp-Zinn, K. (1957) *Indukt. Abstammungs. Verebungsl.* 88:253-285; Napp-Zinn K. (1985) *CRC Handbook of Flowering*, Vol. 1, A. H. Halevy, pp 492-503;  
15 Clarke and Dean (1994) *Mol. Gen. Genet.* 248:81-89; Koornneef. *et al.*, (1994) *Plant Journal* 6:911-919; Lee *et al.*, (1994) *Plant Journal* 6:903-909.) Dominant functional alleles of *FLC* and *FRI* are found together in Northern European *Arabidopsis* ecotypes such as Pitztal and Stockholm. These ecotypes are extremely late flowering when non-vernalized. The widely  
20 used laboratory ecotype Columbia contains functional alleles at only one of these two loci and flower slightly later than strains such as Landsberg *erecta* which possess functional alleles of neither gene. The *FRIGIDA* protein sequence has not yet been published. However, the *FLC* gene has recently been cloned and shown to encode a MADS box protein (Sheldon C. *et al.*, 1999, *Plant Cell* 11:445-458; Michaels S. and Amasino, R., 1999, *Plant Cell* 11:949-956).  
25 Dominant alleles and overexpression of *FLC* have been reported to delay flowering, while null *flc* mutants are early flowering (Lee *et al.*, (1994) *Plant J.* 6:903-909; Michaels and Amasino, (1999) *Plant Cell* 11:949-956; Sheldon *et al.*, (1999) *Proc. Natl. Acad. Sci.* 97:3753-3758). Thus, *FLC* acts to prevent premature flowering.

We have discovered transcription factors that regulate flowering time or vernalization  
30 requirements of plants. These transcription factors could therefore be useful to manipulate flowering characteristics of a plant.

#### SUMMARY OF THE INVENTION

35 In one aspect, the present invention relates to a transgenic plant comprising a recombinant polynucleotide. The recombinant polynucleotide comprises a nucleotide sequence encoding a polypeptide comprising at least 6 consecutive amino acids of a

sequence selected from the group consisting of SEQ ID Nos. 2N, where N=1-28 but excluding SEQ ID No. 28, and the presence of the recombinant polynucleotide alters the flowering time or vernalization requirements of the transgenic plant when compared with the same trait of another plant lacking the recombinant polynucleotide.

5 In one embodiment, the nucleotide sequence encodes a polypeptide comprising a conserved domain such as 1) a localization domain, 2) an activation domain, 3) a repression domain, 4) an oligomerization domain or 5) a DNA binding domain of SEQ ID Nos. 2N, where N=1-28. In another embodiment, the recombinant polynucleotide encodes a polypeptide comprising a conserved domain having greater than an 84% sequence identity to a sequence  
10 selected from the group consisting of SEQ ID Nos. 2N, where N=1-28. In a further embodiment, the nucleotide sequence further comprises a promoter operably linked to the nucleotide sequence. The promoter may be a constitutive or inducible or tissue-active.

In a second aspect, the present invention relates to a method for altering a plant's flowering time or vernalization requirements. The method comprises (a) transforming a plant  
15 with a recombinant polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising at least 6 consecutive amino acids of a sequence selected from the group consisting of SEQ ID Nos. 2N, where N=1-28; (b) selecting transformed plants; and (c) identifying a transformed plant with the desired trait.

In one embodiment, the nucleotide sequence encodes a polypeptide comprising a conserved domain such as 1) a localization domain, 2) an activation domain, 3) a repression domain, 4) an oligomerization domain or 5) a DNA binding domain domain of SEQ ID Nos. 2N, where N=1-28 but excluding SEQ ID No. 28. In another embodiment, the recombinant polynucleotide encodes a polypeptide comprising a conserved domain having greater than an 84% sequence identity to a sequence selected from the group consisting of SEQ ID Nos. 2N,  
20 where N=1-28. In a further embodiment, the nucleotide sequence further comprises a promoter operably linked to the nucleotide sequence. The promoter may be a constitutive or inducible or tissue-active.

In a third aspect, the present invention relates to another method for altering a plant trait associated with flowering time or the plant's vernalization requirements. The method  
30 comprises (a) transforming the plant with a recombinant polynucleotide comprising a nucleotide sequence comprising at least 18 consecutive nucleotides of a sequence selected from the group consisting of SEQ ID Nos. 2N-1, where N= 1-28 but excluding SEQ ID No. 27; and (b) selecting said transformed plant.

In yet another aspect, the present invention is yet another method for altering a plant's  
35 flowering time or vernalization requirements. The method comprises (a) providing a database sequence; (b) comparing the database sequence with a polypeptide selected from SEQ ID Nos. 2N, where N= 1-28; (c) selecting a database sequence that meets selected sequence



criteria; and (d) transforming said database sequence in the plant. Alternatively, the database sequence can be compared with a polynucleotide selected from SEQ ID Nos. 2N-1, where N= 1-28.

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### **BRIEF DESCRIPTION OF THE FIGURES**

Figure 1 provides a table of exemplary polynucleotide and polypeptide sequences of the invention. The table includes from left to right for each sequence: the SEQ ID No., the internal code reference number, whether the sequence is a polynucleotide or polypeptide sequence, and identification of any conserved domains for the polypeptide sequences.

Figure 2 provides a table of sequences that are homologous to the sequences provided in the Sequence Listing. The table includes from left to right: the SEQ ID No., the internal code reference number, the unique Genbank sequence ID No. (NID), the probability that the comparison was generated by chance (P-value), and the species from which the homologous gene was identified.

### **DETAILED DESCRIPTION OF THE INVENTION**

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#### **DEFINITIONS**

A "recombinant polynucleotide" is a nucleotide sequence comprising a gene coding sequence or a fragment thereof (comprising at least 18 consecutive nucleotides, preferably at least 30 consecutive nucleotides, and more preferably at least 50 consecutive nucleotides). Additionally, the polynucleotide may comprise a promoter, an intron, an enhancer region, a polyadenylation site, a translation initiation site, 5' or 3' untranslated regions, a reporter gene, a selectable marker or the like. The polynucleotide may comprise single stranded or double stranded DNA or RNA. The polynucleotide may comprise modified bases or a modified backbone. The polynucleotide may be genomic, a transcript (such as an mRNA) or a processed nucleotide sequence (such as a cDNA). The polynucleotide may comprise a sequence in either sense or antisense orientations.

A "recombinant polynucleotide" is a polynucleotide that is not in its native state, e.g., the polynucleotide is comprised of a nucleotide sequence not found in nature or the polynucleotide is separated from nucleotide sequences with which it typically is in proximity or is next to nucleotide sequences with which it typically is not in proximity.

A "recombinant polypeptide" is a polypeptide derived from the translation of a recombinant polynucleotide or is more enriched in a cell than the polypeptide in its natural

state in a wild type cell, e.g. more than 5% enriched, more than 10% enriched or more than 20% enriched and is not the result of a natural response of a wild type plant or is separated from other components with which it is typically associated with in a cell.

5 A "transgenic plant" may refer to a plant that contains genetic material not normally found in a wild type plant of the same species, or in a naturally occurring variety or in a cultivar, and which has been introduced into the plant by human manipulation. A transgenic plant is a plant that may contain an expression vector or cassette. The expression cassette comprises a gene coding sequence and allows for the expression of the gene coding sequence. The expression cassette may be introduced into a plant by transformation or by  
10 breeding after transformation of a parent plant.

A transgenic plant refers to a whole plant as well as to a plant part, such as seed, fruit, leaf, or root, plant tissue, plant cells, protoplasts or any other plant material, and progeny thereof.

The phrase "altered expression" in reference to polynucleotide or polypeptide  
15 expression refers to an expression pattern in the transgenic plant that is different from the expression pattern in the wild type plant or a reference; for example, by expression in a cell type other than a cell type in which the sequence is expressed in the wild type plant, or by expression at a time other than at the time the sequence is expressed in the wild type plant, or by a response to different inducible agents, such as hormones or environmental signals, or at  
20 different expression levels (either higher or lower) compared with those found in a wild type plant. The term also refers to lowering the levels of expression to below the detection level or completely abolishing expression. The resulting expression pattern may be transient or stable, constitutive or inducible.

A "transcription factor" (TF) refers to a polynucleotide or polypeptide that controls the  
25 expression of a gene or genes either directly by binding to one or more nucleotide sequences associated with a gene coding sequence or indirectly by affecting the level or activity of other polypeptides that do bind directly or indirectly to one or more nucleotide sequences associated with a gene coding sequence. A TF, in this definition, includes any polypeptide that can activate or repress transcription of a single gene or a number of genes. This polypeptide  
30 group includes, but is not limited to, DNA binding proteins, protein kinases, protein phosphatases, GTP-binding proteins and receptors.

The transcription factor sequence may comprise a whole coding sequence or a fragment or domain of a coding sequence. A "fragment or domain", as referred to  
35 polypeptides, may be a portion of a polypeptide which performs at least one biological function of the intact polypeptide in substantially the same manner or to a similar extent as does the intact polypeptide. A fragment may comprise, for example, a DNA binding domain that binds to a specific DNA promoter region, an activation domain or a domain for protein-protein

interactions. Fragments may vary in size from as few as 6 amino acids to the length of the intact polypeptide, but are preferably at least 30 amino acids in length and more preferably at least 60 amino acids in length. In reference to a nucleotide sequence "a fragment" refers to any sequence of at least consecutive 18 nucleotides, preferably at least 30 nucleotides, more preferably at least 50, of any of the sequences provided herein.

Exemplary polynucleotides and polypeptides comprise a sequence provided in the Sequence Listing as SEQ ID No. 1: G157 (cDNA); SEQ ID No. 2: G157 (protein); SEQ ID No. 3: G859 (cDNA); SEQ ID No. 4: G859 (protein); SEQ ID No. 5: G859.1 (cDNA); SEQ ID No. 6: G859.1 (protein); SEQ ID No. 7: G859.2 (cDNA); SEQ ID No. 8: G859.2 (protein); SEQ ID No. 9: G1842 (cDNA); SEQ ID No. 10: G1842 (protein); SEQ ID No. 11: G1842.2 (cDNA); SEQ ID No. 12: G1842.2 (protein); SEQ ID No. 13: G1842.6 (cDNA); SEQ ID No. 14: G1842.6 (protein); SEQ ID No. 15: G1842.7 (cDNA); SEQ ID No. 16: G1842.7 (protein); SEQ ID No. 17: G1843 (cDNA); SEQ ID No. 18: G1843 (protein); SEQ ID No. 19: G1844 (cDNA); SEQ ID No. 20: G1844 (protein); SEQ ID No. 21: G1844.2 (cDNA); SEQ ID No. 22: G1844.2 (protein); SEQ ID No. 23: G861 (cDNA); SEQ ID No. 24: G861 (protein); SEQ ID No. 25: G861.1 (cDNA); SEQ ID No. 26: G861.1 (protein); SEQ ID No. 27: G1759 (cDNA); SEQ ID No. 28: G1759 (protein); SEQ ID No. 29: G192 (cDNA); SEQ ID No. 30: G192 (protein); SEQ ID No. 31: G234 (cDNA); SEQ ID No. 32: G234 (protein); SEQ ID No. 33: G361 (cDNA); SEQ ID No. 34: G361 (protein); SEQ ID No. 35: G486 (cDNA); SEQ ID No. 36: G486 (protein); SEQ ID No. 37: G748 (cDNA); SEQ ID No. 38: G748 (protein); SEQ ID No. 39: G994 (cDNA); SEQ ID No. 40: G994 (protein); SEQ ID No. 41: G1335 (cDNA); SEQ ID No. 42: G1335 (protein); SEQ ID No. 43: G562 (cDNA); SEQ ID No. 44: G562 (protein); SEQ ID No. 45: G736 (cDNA); SEQ ID No. 46: G736 (protein); SEQ ID No. 47: G1073 (cDNA); SEQ ID No. 48: G1073 (protein); SEQ ID No. 49: G1435 (cDNA); SEQ ID No. 50: G1435 (protein); SEQ ID No. 51: G180 (cDNA); SEQ ID No. 52: G180 (protein); SEQ ID No. 53: G592 (cDNA); SEQ ID No. 54: G592 (protein); SEQ ID No. 55: G208 (cDNA); and SEQ ID No. 56: G208 (protein).

A "conserved domain" refers to a polynucleotide or polypeptide fragment that is more conserved at a sequence level than other fragments when the polynucleotide or polypeptide is compared with homologous genes or proteins from other plants. The conserved domain may be 1) a localization domain, 2) an activation domain, 3) a repression domain, 4) a dimerization or oligomerization domain, 5) a DNA binding domain or any combination thereof. For MADS proteins, the conserved domain is typically a DNA-binding domain.

A nucleotide sequence is "operably linked" when it is placed into a functional relationship with another nucleotide sequence. For example, a promoter or enhancer is operably linked to a gene coding sequence if the presence of the promoter or enhancer increases the level of expression of the gene coding sequence.

5 "Trait" refers to a physiological, morphological, biochemical or physical characteristic of a plant or particular plant material or cell. This characteristic may be visible to the human eye, such as seed or plant size, or be measured by biochemical techniques, such as the protein, starch or oil content of seed or leaves or by the observation of the expression level of genes by employing Northernblots, RT PCR, microarray gene expression assays or reporter gene expression systems or be measured by agricultural observations such as stress tolerance, yield or disease resistance.

10 "Trait modification" refers to a detectable difference in a characteristic in a transgenic plant with modified expression of a polynucleotide or polypeptide of the present invention relative to a plant not doing so, such as a wild type plant. The trait modification may entail at least a 5% increase or decrease in an observed trait (difference), at least a 10% difference, at least a 20% difference, at least a 30%, at least a 50%, at least a 70%, at least a 100% or a greater difference. It is known that there may be a natural variation in the modified trait. Therefore, the trait modification observed entails a change in the normal distribution of the trait in transgenic plants compared with the distribution observed in wild type plant.

15 Trait modifications of particular interest include those to seed (embryo), fruit, root, flower, leaf, stem, shoot, seedling or the like, including: enhanced tolerance to environmental conditions including freezing, chilling, heat, drought, water saturation, radiation and ozone; enhanced resistance to microbial, fungal or viral diseases; resistance to nematodes, decreased herbicide sensitivity, enhanced tolerance of heavy metals (or enhanced ability to take up heavy metals), enhanced growth under poor photoconditions (e.g., low light and/or short day length), or changes in expression levels of genes of interest. Other phenotypes that may be modified relate to the production of plant metabolites, such as variations in the production of taxol, tocopherol, tocotrienol, sterols, phytosterols, vitamins, wax monomers, anti-oxidants, amino acids, lignins, cellulose, tannins, prenolipids (such as chlorophylls and carotenoids), glucosinolates, and terpenoids, enhanced or compositionally altered protein or oil production (especially in seeds), or modified sugar (insoluble or soluble) and/or starch composition. Physical plant characteristics that may be modified include cell development (such as the number of trichomes), fruit and seed size and number, yields of plant parts such as stems, leaves and roots, the stability of the seeds during storage, characteristics of the seed pod (e.g., susceptibility to shattering), root hair length and quantity, internode distances, or the quality of seed coat. Plant growth characteristics that may be modified include growth rate, germination rate of seeds, vigor of plants and seedlings, leaf and flower senescence, male sterility, apomixis, flowering time, flower abscission, rate of nitrogen uptake, biomass or transpiration characteristics, as well as plant architecture characteristics such as apical dominance, branching patterns, number of organs, organ identity, organ shape or size.

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Of particular interest are traits relating to modified vernalization requirements or flowering time characteristics, such as changes in flowering time in response to day-length, in response to temperature, in response to light quality, nutrient availability, and development stage of the plant, and the like.

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### 1. The Sequences

We have discovered particular plant transcription factors (TFs) that can be employed to modify the flowering time of a plant. Therefore, the flowering time of plants can be either decreased, increased, or made inducible under specific conditions using the TFs of this invention. Additionally, the transcription factors can be used to modify the vernalization requirements of the plant.

The plant transcription factors may belong to one of the following transcription factor families: the AP2 (APETALA2) domain transcription factor family (Riechmann and Meyerowitz (1998) *Biol. Chem.* 379:633-646); the MYB transcription factor family (Martin and Paz-Ares, (1997) *Trends Genet.* 13:67-73); the MADS domain transcription factor family (Riechmann and Meyerowitz (1997) *Biol. Chem.* 378:1079-1101); the WRKY protein family (Ishiguro and Nakamura (1994) *Mol. Gen. Genet.* 244:563-571); the ankyrin-repeat protein family (Zhang et al. (1992) *Plant Cell* 4:1575-1588); the zinc finger protein (Z) family (Klug and Schwabe (1995) *FASEB J.* 9: 597-604); the homeobox (HB) protein family (Duboule (1994) *Guidebook to the Homeobox Genes*, Oxford University Press); the CAAT-element binding proteins (Forsburg and Guarente (1989) *Genes Dev.* 3:1166-1178); the squamosa promoter binding proteins (SPB) (Klein et al. (1996) *Mol. Gen. Genet.* 1996 250:7-16); the NAM protein family (Souer et al. (1996) *Cell* 85:159-170); the IAA/AUX proteins (Rouse et al. (1998) *Science* 279:1371-1373); the HLH/MYC protein family (Littlewood et al. (1994) *Prot. Profile* 1:639-709); the DNA-binding protein (DBP) family (Tucker et al. (1994) *EMBO J.* 13:2994-3002); the bZIP family of transcription factors (Foster et al. (1994) *FASEB J.* 8:192-200); the Box P-binding protein (the BPF-1) family (da Costa e Silva et al. (1993) *Plant J.* 4:125-135); the high mobility group (HMG) family (Bustin and Reeves (1996) *Prog. Nucl. Acids Res. Mol. Biol.* 54:35-100); the scarecrow (SCR) family (Di Laurenzio et al. (1996) *Cell* 86:423-433); the GF14 family (Wu et al. (1997) *Plant Physiol.* 114:1421-1431); the polycomb (PCOMB) family (Kennison (1995) *Annu. Rev. Genet.* 29:289-303); the teosinte branched (TEO) family (Luo et al. (1996) *Nature* 383:794-799); the ABI3 family (Giraudat et al. (1992) *Plant Cell* 4:1251-1261); the triple helix (TH) family (Dehesh et al. (1990) *Science* 250:1397-1399); the EIL family (Chao et al. (1997) *Cell* 89:1133-44); the AT-HOOK family (Reeves and Nissen (1990) *Journal of Biological Chemistry* 265:8573-8582); the S1FA family (Zhou et al. (1995) *Nucleic Acids Res.* 23:1165-1169); the bZIPT2 family (Lu and Ferl (1995) *Plant Physiol.* 109:723); the YABBY family (Bowman et al. (1999) *Development* 126:2387-96); the PAZ family (Bohmert et al. (1998)

*EMBO J.* 17:170-80); a family of miscellaneous (MISC) transcription factors including the DPBF family (Kim et al. (1997) *Plant J.* 11:1237-1251) and the SPF1 family (Ishiguro and Nakamura (1994) *Mol. Gen. Genet.* 244:563-571); the golden (GLD) family (Hall et al. (1998) *Plant Cell* 10:925-936), and the TUBBY family (Boggin et al. (1999) *Science* 286:2119-2125)

In particular, the TFs that we have discovered that are implicated in flowering time or vernalization include members of the MADS transcription factor family, the MYB family, the WRKY family, the HLH/MYC family, GLD family, AT-HOOK family, the CAAT family, the bZIP family, and members of zinc coordinating protein families (Z-Dof, Z-CLDSH and Z-CH2H2). In fact we have identified the first members of the WRKY, CAAT, bZIP, AT-HOOK and HLH/MYC families that are associated with flowering time modification in plants: G192 and G190 (WRKY), G486 (CAAT), G562 (bZIP), G1073 (AT-HOOK) and G592 (HLH/MYC).

The polynucleotides and polypeptides are provided in the Sequence Listing and are tabulated in Figure 1. Figure 1 identifies a SEQ ID No., its corresponding GID number, whether the sequence is a polynucleotide or a polypeptide sequence, and indicates the conserved domains. We have also identified domains or fragments derived from each of the sequences in the Sequence Listing. The fragments can be from any region of the sequence, can be of any length up to the length of the sequence, and can be as short as six residues for protein and 18 nucleotides for DNA. Exemplary fragments of the DNA sequences are as follows: 1-50, 51-100, 101-200, 201-218, 218-300, 301-450 and 450-600; and exemplary fragments of proteins are as follows 1-50, 51-100, 101-200, 201-206, 206-250, 251-300. For DNA sequences, the numbers may be measured from either 5' or 3' end of the DNA. For the protein sequences the fragment location is determined from the N-terminus or C-terminus of the protein and may include adjacent amino acid sequences, such as for example for SEQ ID No. 2 an additional 10, 20, 40, 60 or 100 amino acids in either N-terminal or C-terminal direction of the described fragments.

The identified polypeptide fragments may be linked to fragments or sequences derived from other transcription factors so as to generate additional novel sequences, such as by employing the methods described in Short, PCT publication WO9827230, entitled "Methods and Compositions for Polypeptide Engineering" or in Patten et al., PCT publication WO9923236, entitled "Method of DNA Shuffling" or in Minshull and Stemmer, US Patent No. 5,837,458. Alternatively, the identified fragment may be linked to a transcription activation domain. A transcription activation domain assists in initiating transcription from a DNA binding site. A common feature of some activation domains is that they are designed to form amphiphilic alpha helices with excess positive or negative charge (Giniger and Ptashne (1987) *Nature* 330:670-672, Gill and Ptashne (1987) *Cell* 51:121-126, Estruch et al (1994) *Nucl. Acids Res.* 22:3983-3989). Examples include the transcription activation region of VP16 or GAL4 (Moore et al. (1998) *Proc. Natl. Acad. Sci. USA* 95: 376-381; and Aoyama et al.

(1995) Plant Cell 7:1773-1785), peptides derived from bacterial sequences (Ma and Ptashne (1987) Cell 51; 113-119) and synthetic peptides (Giniger and Ptashne, supra).

The isolated polynucleotides and polypeptides may be used to modify plant development, physiology or biochemistry such that the modified plants have a trait advantage over wild type plants. The identified polynucleotide fragments are also useful as nucleic acid probes and primers. A nucleic acid probe is useful in hybridization protocols, including protocols for microarray experiments. Primers may be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, and then extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods. See Sambrook et al., *Molecular Cloning. A Laboratory Manual*, Ed. 2, Cold Spring Harbor Laboratory Press, New York (1989) and Ausubel et al. (eds) *Current Protocols in Molecular Biology*, John Wiley & Sons (1998).

## 2. Identification of Homologous Sequences (Homologs)

Homologous sequences to those provided in the Sequence Listing derived from *Arabidopsis thaliana* or from other plants may be used to modify a plant trait. Homologous sequences may be derived from any plant including monocots and dicots and in particular agriculturally important plant species, including but not limited to, crops such as soybean, wheat, corn, potato, cotton, rice, oilseed rape (including canola), sunflower, alfalfa, sugarcane and turf; or fruits and vegetables, such as banana, blackberry, blueberry, strawberry, and raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits (such as apple, peach, pear, cherry and plum) and vegetable brassicas (such as broccoli, cabbage, cauliflower, brussel sprouts and kohlrabi). Other crops, fruits and vegetables whose phenotype may be changed include barley, currant, avocado, citrus fruits such as oranges, lemons, grapefruit and tangerines, artichoke, cherries, nuts such as the walnut and peanut, endive, leek, roots, such as arrowroot, beet, cassava, turnip, radish, yam, sweet potato and beans. The homologs may also be derived from woody species, such as pine, poplar and eucalyptus.

Substitutions, deletions and insertions introduced into the sequences provided in the Sequence Listing are also envisioned by the invention. Such sequence modifications can be engineered into a sequence by site-directed mutagenesis (Wu (ed.) *Meth. Enzymol.* (1993) vol. 217, Academic Press). Amino acid substitutions are typically of single residues; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. In preferred embodiments, deletions or insertions are

made in adjacent pairs, e.g., a deletion of two residues or insertion of two residues. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a sequence. The mutations that are made in the polynucleotide encoding the transcription factor should not place the sequence out of reading frame and should not create complementary regions that could produce secondary mRNA structure.

Substitutions are those in which at least one residue in the amino acid sequence has been removed and a different residue inserted in its place. Such substitutions may be conservative with little effect on the function of the gene, for example by substituting alanines for serines, arginines for lysines, glutamate for aspartate and the like. The substitutions which are not conservative are expected to produce the greatest changes in protein properties will be those in which (a) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine.

Additionally, the term "homologous sequence" may encompass a polypeptide sequence that is modified by chemical or enzymatic means. The homologous sequence may be a sequence modified by lipids, sugars, peptides, organic or inorganic compounds, by the use of modified amino acids or the like. Protein modification techniques are illustrated in Ausubel et al. (eds) *Current Protocols in Molecular Biology*, John Wiley & Sons (1998).

Homologous sequences also may mean two sequences having a substantial percentage of sequence identity after alignment as determined by using sequence analysis programs for database searching and sequence alignment and comparison available, for example, from the Wisconsin Package Version 10.0, such as BLAST, FASTA, PILEUP, FINDPATTERNS or the like (GCG, Madison, WI). Public sequence databases such as GenBank, EMBL, Swiss-Prot and PIR or private sequence databases such as PhytoSeq (Incyte Pharmaceuticals, Palo Alto, CA) may be searched. Alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman (1981) *Adv. Appl. Math.* 2:482, by the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443, by the search for similarity method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. U.S.A.* 85: 2444, by computerized implementations of these algorithms. After alignment, sequence comparisons between two (or more) polynucleotides or polypeptides are typically performed by comparing sequences of the two sequences over a comparison window to identify and compare local regions of sequence similarity. The comparison window may be a segment of at least about 20 contiguous positions, usually about 50 to about 200, more usually about 100 to about 150 contiguous



positions. A description of the method is provided in Ausubel et al. (eds) (1999) *Current Protocols in Molecular Biology*, John Wiley & Sons.

Transcription factors that are homologs of the disclosed sequences will typically share at least 40% amino acid sequence identity. More closely related TFs may share at least 50%,  
5 60%, 65%, 70%, 75% or 80% sequence identity with the disclosed sequences. Factors that are most closely related to the disclosed sequences share at least 85%, 90% or 95% sequence identity. At the nucleotide level, the sequences will typically share at least 40% nucleotide sequence identity, preferably at least 50%, 60%, 70% or 80% sequence identity, and more preferably 85%, 90%, 95% or 97% sequence identity. The degeneracy of the  
10 genetic code enables major variations in the nucleotide sequence of a polynucleotide while maintaining the amino acid sequence of the encoded protein.

One way to identify whether two nucleic acid molecules are closely related is that the two molecules hybridize to each other under stringent conditions. Generally, stringent conditions are selected to be about 5°C to 20°C lower than the thermal melting point ( $T_m$ ) for the specific  
15 sequence at a defined ionic strength and pH. The  $T_m$  is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Conditions for nucleic acid hybridization and calculation of stringencies can be found in Sambrook et al. (1989) *Molecular Cloning. A Laboratory Manual*, Ed. 2, Cold Spring Harbor Laboratory Press, New York and Tijssen (1993) *Laboratory Techniques in Biochemistry and  
20 Molecular Biology--Hybridization with Nucleic Acid Probes Part I*, Elsevier, New York. Nucleic acid molecules that hybridize under stringent conditions will typically hybridize to a probe based on either the entire cDNA or selected portions of the cDNA under wash conditions of 0.2x SSC to 2.0 x SSC, 0.1% SDS at 50-65° C, for example 0.2 x SSC, 0.1% SDS at 65° C. For detecting less closely related homologs washes may be performed at 50° C.

For conventional hybridization the hybridization probe is conjugated with a detectable  
25 label such as a radioactive label, and the probe is preferably of at least 20 nucleotides in length. As is well known in the art, increasing the length of hybridization probes tends to give enhanced specificity. The labeled probe derived from the *Arabidopsis* nucleotide sequence may be hybridized to a plant cDNA or genomic library and the hybridization signal detected  
30 using means known in the art. The hybridizing colony or plaque (depending on the type of library used) is then purified and the cloned sequence contained in that colony or plaque isolated and characterized. Homologs may also be identified by PCR-based techniques, such as inverse PCR or RACE, using degenerate primers. See Ausubel et al. (eds) (1998) *Current Protocols in Molecular Biology*, John Wiley & Sons.

35 TF homologs may alternatively be obtained by immunoscreening an expression library. With the provision herein of the disclosed TF nucleic acid sequences, the polypeptide may be expressed and purified in a heterologous expression system (e.g., *E. coli*) and used to raise

antibodies (monoclonal or polyclonal) specific for the TF. Antibodies may also be raised against synthetic peptides derived from TF amino acid sequences. Methods of raising antibodies are well known in the art and are described in Harlow and Lane (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York. Such antibodies can then be used to screen an expression library produced from the plant from which it is desired to clone the TF homolog, using the methods described above. The selected cDNAs may be confirmed by sequencing and biological activity.

### 3. Altered Expression of Transcription Factors

Any of the identified sequences may be incorporated into a cassette or vector for expression in plants. A number of expression vectors suitable for stable transformation of plant cells or for the establishment of transgenic plants have been described including those described in Weissbach and Weissbach, (1989) *Methods for Plant Molecular Biology*, Academic Press, and Gelvin et al., (1990) *Plant Molecular Biology Manual*, Kluwer Academic Publishers. Specific examples include those derived from a Ti plasmid of *Agrobacterium tumefaciens*, as well as those disclosed by Herrera-Estrella, L., et al., (1983) *Nature* 303: 209, Bevan, M., *Nucl. Acids Res.* (1984) 12: 8711-8721, Klee, H. J., (1985) *Bio/Technology* 3: 637-642, for dicotyledonous plants. Ti-derived plasmids can be transferred into both monocotyledonous and dicotyledonous species using *Agrobacterium*-mediated transformation (Ishida et al (1996) *Nat. Biotechnol.* 14:745-50; Barton et al. (1983) *Cell* 32:1033-1043).

Alternatively, non-Ti vectors can be used to transfer the DNA into plants and cells by using free DNA delivery techniques. Such methods may involve, for example, the use of liposomes, electroporation, microprojectile bombardment, silicon carbide whiskers, and viruses. By using these methods transgenic plants such as wheat, rice (Christou, P., (1991) *Bio/Technology* 9: 957-962) and corn (Gordon-Kamm, W., (1990) *Plant Cell* 2: 603-618) can be produced. An immature embryo can also be a good target tissue for monocots for direct DNA delivery techniques by using the particle gun (Weeks, T. et al., (1993) *Plant Physiol.* 102: 1077-1084; Vasil, V., (1993) *Bio/Technology* 10: 667-674; Wan, Y. and Lemeaux, P., (1994) *Plant Physiol.* 104: 37-48, and for *Agrobacterium*-mediated DNA transfer (Ishida et al., (1996) *Nature Biotech.* 14: 745-750).

Typically, plant transformation vectors include one or more cloned plant coding sequences (genomic or cDNA) under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant transformation vectors typically also contain a promoter (e.g., a regulatory region controlling inducible or constitutive, environmentally-or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, an RNA processing signal (such as intron splice sites), a transcription termination site, and/or a polyadenylation signal.

Examples of constitutive plant promoters which may be useful for expressing the TF sequence include: the cauliflower mosaic virus (CaMV) 35S promoter, which confers constitutive, high-level expression in most plant tissues (see, e.g., Odel et al., (1985) *Nature* 313:810); the nopaline synthase promoter (An et al., (1988) *Plant Physiol.* 88:547); and the octopine synthase promoter (Fromm et al., (1989) *Plant Cell* 1: 977).

A variety of plant gene promoters that regulate gene expression in response to environmental, hormonal, chemical, developmental signals, and in a tissue-active manner can be used for expression of the TFs in plants, as illustrated by seed-specific promoters (such as the napin, phaseolin or DC3 promoter described in US Pat. No. 5,773,697), root-specific promoters, such as those disclosed in US Patent Nos. 5,618,988, 5,837,848 and 5,905,186; fruit-specific promoters that are active during fruit ripening (such as the dru 1 promoter (US Pat. No. 5,783,393), or the 2A11 promoter (US Pat. No. 4,943,674) and the tomato polygalacturonase promoter (Bird et al. (1988) *Plant Mol. Biol.* 11:651), root-specific promoters, such as those disclosed in US Patent Nos. 5,618,988, 5,837,848 and 5,905,186, pollen-active promoters such as PTA29, PTA26 and PTA13 (US Pat. No. 5,792,929), promoters active in vascular tissue (Ringli and Keller (1998) *Plant Mol. Biol.* 37:977-988), flower-specific (Kaiser et al., (1995) *Plant Mol. Biol.* 28:231-243), pollen (Baerson et al. (1994) *Plant Mol. Biol.* 26:1947-1959), carpels (Ohl et al. (1990) *Plant Cell* 2:837-848), pollen and ovules (Baerson et al. (1993) *Plant Mol. Biol.* 22:255-267), auxin-inducible promoters (such as that described in van der Kop et al (1999) *Plant Mol. Biol.* 39:979-990 or Baumann et al. (1999) *Plant Cell* 11:323-334), cytokinin-inducible promoter (Guevara-Garcia (1998) *Plant Mol. Biol.* 38:743-753), promoters responsive to gibberellin (Shi et al. (1998) *Plant Mol. Biol.* 38:1053-1060, Willmott et al. (1998) 38:817-825) and the like. Additional promoters are those that elicit expression in response to heat (Ainley, et al. (1993) *Plant Mol. Biol.* 22: 13-23), light (e.g., the pea rbcS-3A promoter, Kuhlemeier et al., (1989) *Plant Cell* 1:471, and the maize rbcS promoter, Schaffner and Sheen, (1991) *Plant Cell* 3: 997); wounding (e.g., *wun1*, Siebertz et al., (1989) *Plant Cell* 1: 961); pathogen resistance, and chemicals such as methyl jasmonate or salicylic acid. (Gatz et al., (1997) *Plant Mol. Biol.* 48: 89-108). In addition, the timing of the expression can be controlled by using promoters such as those acting at late seed development (Odell et al. (1994) *Plant Physiol.* 106:447-458).

Plant expression vectors may also include RNA processing signals that may be positioned within, upstream or downstream of the coding sequence. In addition, the expression vectors may include additional regulatory sequences from the 3'-untranslated region of plant genes, e.g., a 3' terminator region to increase mRNA stability of the mRNA, such as the PI-II terminator region of potato or the octopine or nopaline synthase 3' terminator regions.

Finally, as noted above, plant expression vectors may also include dominant selectable marker genes to allow for the ready selection of transformants. Such genes include those encoding antibiotic resistance genes (e.g., resistance to hygromycin, kanamycin, bleomycin, G418, streptomycin or spectinomycin) and herbicide resistance genes (e.g., phosphinothricin acetyltransferase).

A reduction of TF expression in a transgenic plant to modify a plant trait may be obtained by introducing into plants antisense constructs based on the TF cDNA. For antisense suppression, the TF cDNA is arranged in reverse orientation relative to the promoter sequence in the expression vector. The introduced sequence need not be the full length TF cDNA or gene, and need not be identical to the TF cDNA or a gene found in the plant type to be transformed. Generally, however, where the introduced sequence is of shorter length, a higher degree of homology to the native TF sequence will be needed for effective antisense suppression. Preferably, the introduced antisense sequence in the vector will be at least 30 nucleotides in length, and improved antisense suppression will typically be observed as the length of the antisense sequence increases. Preferably, the length of the antisense sequence in the vector will be greater than 100 nucleotides. Transcription of an antisense construct as described results in the production of RNA molecules that are the reverse complement of mRNA molecules transcribed from the endogenous TF gene in the plant cell. Suppression of endogenous TF gene expression can also be achieved using a ribozyme. Ribozymes are synthetic RNA molecules that possess highly specific endoribonuclease activity. The production and use of ribozymes are disclosed in U.S. Patent No. 4,987,071 to Cech and U.S. Patent No. 5,543,508 to Haselhoff. The inclusion of ribozyme sequences within antisense RNAs may be used to confer RNA cleaving activity on the antisense RNA, such that endogenous mRNA molecules that bind to the antisense RNA are cleaved, which in turn leads to an enhanced antisense inhibition of endogenous gene expression.

Vectors in which RNA encoded by the TF cDNA (or variants thereof) is over-expressed may also be used to obtain co-suppression of the endogenous TF gene in the manner described in U.S. Patent No. 5,231,020 to Jorgensen. Such co-suppression (also termed sense suppression) does not require that the entire TF cDNA be introduced into the plant cells, nor does it require that the introduced sequence be exactly identical to the endogenous TF gene. However, as with antisense suppression, the suppressive efficiency will be enhanced as (1) the introduced sequence is lengthened and (2) the sequence similarity between the introduced sequence and the endogenous TF gene is increased.

Vectors expressing an untranslatable form of the TF mRNA may also be used to suppress the expression of endogenous TF activity to modify a trait. Methods for producing such constructs are described in U.S. Patent No. 5,583,021 to Dougherty et al. Preferably, such constructs are made by introducing a premature stop codon into the TF gene. Alternatively, a

plant trait may be modified by gene silencing using double-strand RNA (Sharp (1999) *Genes and Development* 13: 139-141). This approach, whereby a vector is prepared in which a cDNA or gene is arranged in duplicated fashion and is capable of generating upon expression a double stranded RNA molecule with a hairpin structure. This procedure has been used to modify gene activity in plants (Chuang and Meyerowitz (1999) *Proc. Natl. Acad. Sci.* 97:4985-9490).

Another method for abolishing the expression of a gene is by insertion mutagenesis using the T-DNA of *Agrobacterium tumefaciens*. After generating the insertion mutants, the mutants can be screened to identify those containing the insertion in a TF gene. Mutants containing a single mutation event at the desired gene may be crossed to generate homozygous plants for the mutation (Koncz et al. (1992) *Methods in Arabidopsis Research*. World Scientific).

A plant trait may also be modified by using the cre-lox system (for example, as described in US Pat. No. 5,658,772). A plant genome may be modified to include first and second lox sites that are then contacted with a Cre recombinase. If the lox sites are in the same orientation, the intervening DNA sequence between the two sites is excised. If the lox sites are in the opposite orientation, the intervening sequence is inverted.

The polynucleotides and polypeptides of this invention may also be expressed in a plant in the absence of an expression cassette by manipulating the activity or expression level of the endogenous gene by other means. For example, by ectopically expressing a gene by T-DNA activation tagging (Ichikawa et al., (1997) *Nature* 390 698-701, Kakimoto et al., (1996) *Science* 274: 982-985). This method entails transforming a plant with a gene tag containing multiple transcriptional enhancers and once the tag has inserted into the genome, expression of a flanking gene coding sequence becomes deregulated. In another example, the transcriptional machinery in a plant may be modified so as to increase transcription levels of a polynucleotide of the invention (See PCT Publications WO9606166 and WO 9853057 which describe the modification of the DNA binding specificity of zinc finger proteins by changing particular amino acids in the DNA binding motif).

The transgenic plant may also comprise the machinery necessary for expressing or altering the activity of a polypeptide encoded by an endogenous gene, for example by altering the phosphorylation state of the polypeptide to maintain it in an activated state.

#### 4. Transgenic Plants with Modified TF Expression

Once an expression cassette comprising a polynucleotide encoding a TF gene of this invention has been constructed, standard techniques may be used to introduce the polynucleotide into a plant in order to modify a trait of the plant. The plant may be any higher plant, including gymnosperms, monocotyledonous and dicotyledonous plants. Suitable protocols are available for *Leguminosae* (alfalfa, soybean, clover, etc.), *Umbelliferae* (carrot, celery, parsnip), *Cruciferae* (cabbage, radish, rapeseed, broccoli, etc.), *Curcubitaceae*

(melons and cucumber), *Gramineae* (wheat, corn, rice, barley, millet, etc.), *Solanaceae* (potato, tomato, tobacco, peppers, etc.), and various other crops. See protocols described in Ammirato et al. (1984) *Handbook of Plant Cell Culture—Crop Species*. Macmillan Publ. Co. Shimamoto et al. (1989) *Nature* 338:274-276; Fromm et al. (1990) *Bio/Technology* 8:833-839; and Vasil et al. (1990) *Bio/Technology* 8:429-434.

Transformation and regeneration of both monocotyledonous and dicotyledonous plant cells is now routine, and the selection of the most appropriate transformation technique will be determined by the practitioner. The choice of method will vary with the type of plant to be transformed; those skilled in the art will recognize the suitability of particular methods for given plant types. Suitable methods may include, but are not limited to: electroporation of plant protoplasts; liposome-mediated transformation; polyethylene glycol (PEG) mediated transformation; transformation using viruses; micro-injection of plant cells; micro-projectile bombardment of plant cells; vacuum infiltration; and *Agrobacterium tumefaciens* mediated transformation. Transformation means introducing a nucleotide sequence in a plant in a manner to cause stable or transient expression of the sequence.

Successful examples of the modification of plant characteristics by transformation with cloned sequences which serve to illustrate the current knowledge in this field of technology, and which are herein incorporated by reference, include: U.S. Patent Nos. 5,571,706; 5,677,175; 5,510,471; 5,750,386; 5,597,945; 5,589,615; 5,750,871; 5,268,526; 5,780,708; 5,538,880; 5,773,269; 5,736,369 and 5,610,042.

Following transformation, plants are preferably selected using a dominant selectable marker incorporated into the transformation vector. Typically, such a marker will confer antibiotic or herbicide resistance on the transformed plants, and selection of transformants can be accomplished by exposing the plants to appropriate concentrations of the antibiotic or herbicide.

After transformed plants are selected and grown to maturity, those plants showing a modified trait are identified. The modified trait may be any of those traits described above. Additionally, to confirm that the modified trait is due to changes in expression levels or activity of the polypeptide or polynucleotide of the invention may be determined by analyzing mRNA expression using Northern blots, RT-PCR or microarrays, or protein expression using immunoblots or Western blots or gel shift assays.

## 5. Commercial Applications of the Polynucleotides and Polypeptides

Specific applications for the genes of the present invention relate to their potential roles in plant flowering time or the vernalization response. Most modern crop varieties are the result of extensive breeding programs and many generations of backcrossing may be required

to introduce desired traits. Systems that accelerate flowering could have valuable applications in such programs since they allow much faster generation times. Additionally, in some instances, a faster generation time might allow additional harvests of a crop to be made within a given growing season. With the advent of transformation systems for tree species such as oil palm, aspen, pine and eucalyptus, forest biotechnology is a growing area of interest.

Also, in species such as sugarbeet where the vegetative parts of the plants constitute the crop and the reproductive tissues are discarded, it would be advantageous to delay or prevent flowering. Extending vegetative development could bring about large increases in yields.

Furthermore, by regulating the expression of flowering-time controlling genes, using inducible promoters, flowering could potentially be triggered as desired (for example, by application of a chemical inducer). This would allow, for example, flowering to be synchronized across a crop and facilitate more efficient harvesting. Such inducible systems could be used to tune the flowering of crop varieties to different latitudes. At present, species such as soybean and cotton are available as a series of maturity groups that are suitable for different latitudes on the basis of their flowering time (which is governed by day-length). A system in which flowering could be chemically controlled would allow a single high-yielding northern maturity group to be grown at any latitude. In southern regions such plants could be grown for longer, thereby increasing yields, before flowering was induced. In more northern areas, the induction would be used to ensure that the crop flowers prior to the first winter frosts. Currently, the existence of a series of maturity groups for different latitudes represents a major barrier to the introduction of new valuable traits.

For many crop species, high yielding winter-varieties can only be grown in temperate regions where the winter season is prolonged and cold enough to elicit a vernalization response. Altered expression of the genes of the invention could compensate for a vernalization treatment in late-flowering *Arabidopsis* ecotypes. Similar effects might be achieved in crop plants. Winter varieties of wheat, for instance, which over-express G157 (or the wheat ortholog) might then be grown in areas like Southern California which would otherwise be too warm to allow effective vernalization. A second application for this system is in cherry (*Prunus*). Locally grown cherries are unavailable in the early Californian spring since the winters are too warm for vernalization to occur.

A further application exists in strawberry (*Fragaria*). Strawberry has a well-defined perennial cycle of flower initiation, dormancy, chilling, crop growth and runner production. In temperate European countries, the plants flower in early spring, and fruit is produced in May or June. Following fruiting, runners are generated that carry plantlets which take root. The plants then remain dormant all through the late summer and autumn. Flowering cannot be repeated until the following spring after the plants have received a winter cold treatment. A

system, which bypasses this vernalization requirement, might permit a second autumn crop of strawberries to be harvested in addition to the spring crop.

Finally, in addition to the direct applications of the genes themselves, their regulatory regions could also be of value. If the promoters of these genes are responsive to low temperatures they could be incorporated into expression systems for regulation of genes that confer tolerance to freezing. Such genes would then be up regulated specifically at the time required, thereby minimizing any toxic effects that result from their constitutive expression.

## 6. Other Utility of the Polypeptide and Polynucleotides

A transcription factor coding provided by the present invention may also be used to identify exogenous or endogenous molecules that may affect expression of the transcription factors and may affect flowering time. These molecules may include organic or inorganic compounds.

For example, the method may entail first placing the molecule in contact with a plant or plant cell. The molecule may be introduced by topical administration, such as spraying or soaking of a plant, and then the molecule's effect on the expression or activity of the TF polypeptide or the expression of the polynucleotide monitored. Changes in the expression of the TF polypeptide may be monitored by use of polyclonal or monoclonal antibodies, gel electrophoresis or the like. Changes in the expression of the corresponding polynucleotide sequence may be detected by use of microarrays, Northern blots or any other technique for monitoring changes in mRNA expression. These techniques are exemplified in Ausubel et al. (eds) *Current Protocols in Molecular Biology*, John Wiley & Sons (1998). Such changes in the expression levels may be correlated with modified plant traits and thus identified molecules may be useful for soaking or spraying on fruit, vegetable and grain crops to modify traits in plants.

The transcription factors may also be employed to identify promoter sequences with which they may interact. After identifying a promoter sequence, interactions between the transcription factor and the promoter sequence may be modified by changing specific nucleotides in the promoter sequence or specific amino acids in the transcription factor that interact with the promoter sequence to alter a plant trait. Typically, transcription factor DNA binding sites are identified by gel shift assays. After identifying the promoter regions, the promoter region sequences may be employed in double-stranded DNA arrays to identify molecules that affect the interactions of the TFs with their promoters (Bulyk et al. (1999) *Nature Biotechnology* 17:573-577).

The identified transcription factors are also useful to identify proteins that modify the activity of the transcription factor. Such modification may occur by covalent modification, such



as by phosphorylation, or by protein-protein (homo or-heteropolymer) interactions. Any method suitable for detecting protein-protein interactions may be employed. Among the methods that may be employed are co-immunoprecipitation, cross-linking and co-purification through gradients or chromatographic columns, and the two-hybrid yeast system.

5           The two-hybrid system detects protein interactions in vivo and is described in Chien, et al., (1991), *Proc. Natl. Acad. Sci. USA*, 88, 9578-9582 and is commercially available from Clontech (Palo Alto, Calif.). In such a system, plasmids are constructed that encode two hybrid proteins: one consists of the DNA-binding domain of a transcription activator protein fused to the TF polypeptide and the other consists of the transcription activator protein's  
10           activation domain fused to an unknown protein that is encoded by a cDNA that has been recombined into the plasmid as part of a cDNA library. The DNA-binding domain fusion plasmid and the cDNA library are transformed into a strain of the yeast *Saccharomyces cerevisiae* that contains a reporter gene (e.g., lacZ) whose regulatory region contains the transcription activator's binding site. Either hybrid protein alone cannot activate transcription of  
15           the reporter gene. Interaction of the two hybrid proteins reconstitutes the functional activator protein and results in expression of the reporter gene, which is detected by an assay for the reporter gene product. Then, the library plasmids responsible for reporter gene expression are isolated and sequenced to identify the proteins encoded by the library plasmids. After  
20           identifying proteins that interact with the transcription factors, assays for compounds that interfere with the TF protein-protein interactions may be preformed.

The following examples are intended to illustrate but not limit the present invention.

## EXAMPLES

### Methods

25           All experiments were performed using *Arabidopsis* of ecotype Columbia except where otherwise indicated. The Stockholm (CS6863) and Pitztal (CS6832) lines were supplied by the ABRC at Ohio State University. In all experiments, seeds were sterilized by a 2 minute ethanol treatment followed by 30 minutes in 30% bleach / 0.01% Tween and five washes in distilled water. Seeds were sown to MS agar in 0.1% agarose and stratified for 3-5 days at 4  
30           °C, before transfer to growth rooms with a temperature of 20-25 °C. MS media was supplemented with 50mg/l kanamycin for selection of transformed plants. Plants were transplanted to soil after 7 days of growth on plates. For vernalization treatments, seeds were sown to MS agar plates, sealed with micropore tape, and placed in a 4°C cold room with low light levels for 6-8 weeks. The plates were then transferred to the growth rooms alongside  
35           plates containing freshly sown non-vernalized controls. Whole vegetative seedlings were harvested for gene expression analysis at 6 to 9 days after transfer. Rosette leaves were counted when a visible inflorescence of approximately 3 cm was apparent. Rosette and total

leaf number on the progeny stem are tightly correlated with the timing of flowering (Koorneef et al (1991) *Mol. Gen. Genet* 229:57-66.

#### Example I. Full Length Gene Identification and Cloning

5 For the following examples, G157 refers to SEQ ID Nos 1 and 2, G859 refers to SEQ ID Nos. 3-8, G1842 refers to SEQ ID Nos. 9-16, G1843 refers to SEQ ID Nos. 17 and 18, G1844 refers to SEQ ID Nos. 19-22, G861 refers to SEQ ID Nos. 23-26 and FLC or G1759 refers to SEQ ID Nos. 27, 28.

10 Putative transcription factor sequences (genomic or ESTs) related to known transcription factors were identified in the *Arabidopsis thaliana* GenBank database using the tblastn sequence analysis program using default parameters and a P-value cutoff threshold of -4 or -5 or lower, depending on the length of the query sequence. Putative transcription factor sequence hits were then screened to identify those containing particular sequence strings. If the sequence hits contained such sequence strings, the sequences were confirmed  
15 as transcription factors.

For example, we identified a MADS box gene G157 within BAC F22K20 (GenBank accession AC002291) from Chromosome 1 that was predicted to encode a protein related to FLC. An 872bp cDNA clone for G157 was identified among clones isolated from a library derived from leaf mRNA. The encoded protein was 196 amino acids in length, and shared  
20 62% overall amino acid sequence identity with FLC, and 82% identity within the MADS DNA binding domain.

G157 is also related to G859, G1842, G1843, and G1844 that map together as a tightly linked cluster, at the bottom of chromosome V, that occupies approximately 22 kb and spans three adjacent clones, MXK3, F15O5, and MQN23 (GenBank accession numbers  
25 AB019236, AB026633, and AB013395, respectively). G859, G1842, G1843, and G1844 are all arranged in the same orientation. G859, G1842, G1843, and G1844 were likely created by a duplication event; this could have allowed their divergence into different aspects of gene regulation. Their physical proximity suggests that they may act as a unit controlled via common regulatory elements.

30 The pair-wise comparisons of the 57 amino acid MADS domains of FLC, G157, G859, G1842, G1843, and G1844 are displayed in Table 1. The table shows percent amino acid sequence identity and, in parentheses, the sequence identity percentages when conservative amino acid substitutions are considered. The MADS domains of the proteins encoded by G859, G1842, G1843, and G1844 are highly conserved with those of FLC and G157: these  
35 proteins share from 75% to 91% of amino acid sequence identity, depending on the pair-wise comparison as shown below. When conservative amino substitutions are made, the MADS domains of these proteins are 88%-99% identical to each other (shown in parentheses).

Table 1 Percentage of amino acid identity in the MADS domain

	FLC (G1759)	G157	G859	G1842	G1843	G1844
FLC (G1759)	100%	82%(96%)	84%(94%)	77%(91%)	78%(99%)	75%(92%)
G157	-	100%	87%(95%)	89%(94%)	78%(95%)	78%(93%)
G859	-	-	100%	91%(94%)	77%(94%)	78%(92%)
G1842	-	-	-	100%	77%(91%)	78%(88%)
G1843	-	-	-	-	100%	85%(92%)
G1844	-	-	-	-	-	100%

5

Amino acid residue 30 of FLC and by G157, G859, G1842, G1843, and G1844 is an acidic residue (E or D) whereas, in all other *Arabidopsis* MADS domain proteins so far identified, that position is occupied by a positively charged lysine residue. The crystal structure of the human SRF MADS domain bound to DNA has shown that lysine residue (which is also conserved in yeast MCM1 and human MEF2A proteins) to contact the phosphate backbone of the DNA target site (Pellegrini *et al.*, (1995) Nature 376:490-498). That amino acid difference could therefore confer DNA binding properties to FLC and by G157, G859, G1842, G1843, and G1844 distinct from other *Arabidopsis* MADS domain proteins. Therefore, MADS domain proteins with an acidic residue at position 30 may be particularly useful in modifying plant flowering time and vernalization response.

15

The transcripts from these genes were analyzed by 3' RACE (Rapid Amplification cDNA Ends) and corresponding cDNAs were isolated by RT-PCR from mixed samples of *Arabidopsis* tissue (Columbia ecotype). During this analysis, it was found that G859, G1842 and G1844 transcripts exist in multiple alternatively spliced forms.

20

#### Example II. Flowering Time Associated Genes

Reverse transcriptase PCR was done using gene specific primers within the coding region for each sequence identified. Where possible, the primers were designed near the 3' region of each coding sequence initially identified.

25

Total RNA was isolated from plant tissue and extracted using CTAB. Once extracted total RNA was normalized in concentration across all the tissue types to ensure that the PCR reaction for each tissue received the same amount of cDNA template using the 28S band as reference. Poly A+ was purified using a modified protocol from the Qiagen Oligotex kit batch protocol. cDNA was synthesized using standard protocols. After the first strand cDNA synthesis, primers for Actin 2 were used to normalize the concentration of cDNA across the tissue types. Actin 2 is found to be constitutively expressed in fairly equal levels across the *Arabidopsis* tissue types.

30

For RT PCR, cDNA template was mixed with corresponding primers and Taq polymerase. Each reaction consisted of 0.2 ul cDNA template, 2ul 10X Tricine buffer, and

16.8 ul water, 5pmol Primer 1, 5pmol Primer 2, 0.3 ul Taq polymerase, 200uM dNTPs and 8.6 ul water.

The 96 well plate was covered with microfilm and set in the Thermocycler to start the following reaction cycle. Step1 93° C for 3 mins, Step 2 93° C for 30 sec, Step 3 60-65° C for 1 min, Step 4 72° C for 2 mins,. Steps 2, 3 and 4 were repeated for 20-35 cycles, Step 5 72° C for 5 mins and Step 6 4° C. The PCR plate was sometimes placed back in the thermocycler to amplify more products for 5-15 more cycles to identify genes that have very low expression. The reaction cycle was as follows: Step 2 93° C for 30 sec, Step 3 65° C for 1 min, and Step 4 72° C for 2 ins, repeated for 8 cycles, and Step 4 4° C.

Eight microliters of PCR product and 1.5 ul of loading dye were loaded on a 1.2% agarose gel for analysis between 21 and 36 cycles. Expression levels of specific transcripts were considered low if they were only detectable after 35 cycles of PCR. Expression levels were considered medium or high depending on the levels of transcript compared with observed transcript levels for actin2.

As an example, to assess G157 mRNA levels in G157 plants, PCR was carried out over 25 cycles using primers 5'-GGCATAACCCTTATCGGAGATTGAAGC-3' (SEQ ID No. 57) and 5'-ACACAACTCTGATCTTGTCTCCGAAGG-3' (SEQ ID No. 58). To assess mRNA levels in different tissues extracted from wild type plants, 25 or 30 cycles of PCR were performed using primers 5'-GCATAACCCTTATCGGAGATTGAAGCCAT-3' (SEQ ID No. 59) and 5'-AACATTCCTCTCTCATCATCTGTTGCCAGC-3' (SEQ ID No. 60). PCR for *FLC* was performed either with primers 5'-AACGCTTAGTATCTCCGGCGACTTGAAC-3' (SEQ ID No. 51) and 5'-CTCACACGAATAAGGTACAAAGTTCATC-3' (SEQ ID No. 62) over 35 cycles, or 5'-TTAGTATCTCCGGCGACTTGAACCCAAACC-3' (SEQ ID No. 63) and 5'-AGATTCTCAACAAGCTTCAACATGAGTTTCG-3' (SEQ ID No. 64) over 30 cycles. Primer specificity was verified by sequencing RT-PCR products. Samples were standardized via 20-25 cycles of PCR with actin primers.

### Example III. Construction of Expression Vectors

The sequence was amplified from a genomic or cDNA library using primers specific to sequences upstream and downstream of the coding region. The expression vector was pMEN20 or pMEN65, which are both derived from pMON316 (Sanders et al, (1987) *Nucleic Acids Research* 15:1543-58) and contain the CaMV 35S promoter to express transgenes. To clone the sequence into the vector, both pMEN20 and the amplified DNA fragment were digested separately with Sall and NotI restriction enzymes at 37° C for 2 hours. The digestion products were subject to electrophoresis in a 0.8% agarose gel and visualized by ethidium bromide staining. The DNA fragments containing the sequence and the linearized plasmid

were excised and purified by using a Qiaquick gel extraction kit (Qiagen, CA). The fragments of interest were ligated at a ratio of 3:1 (vector to insert). Ligation reactions using T4 DNA ligase (New England Biolabs, MA) were carried out at 16° C for 16 hours. The ligated DNAs were transformed into competent cells of the *E. coli* strain DH5alpha by using the heat shock method. The transformations were plated on LB plates containing 50 mg/l spectinomycin (Sigma).

Individual colonies were grown overnight in five milliliters of LB broth containing 50 mg/l spectinomycin at 37° C. Plasmid DNA was purified by using Qiaquick Mini Prep kits (Qiagen, CA).

#### Example IV. Transformation of *Agrobacterium* with the Expression Vector

After the plasmid vector containing the gene was constructed, the vector was used to transform *Agrobacterium tumefaciens* cells expressing the gene products. The stock of *Agrobacterium tumefaciens* cells for transformation were made as described by Nagel et al. *FEMS Microbiol Letts* 67: 325-328 (1990). *Agrobacterium* strain GV3101 was grown in 250 ml LB medium (Sigma) overnight at 28°C with shaking until an absorbance ( $A_{600}$ ) of 0.5 – 1.0 was reached. Cells were harvested by centrifugation at 4,000 x g for 15 min at 4° C. Cells were then resuspended in 250 µl chilled buffer (1 mM HEPES, pH adjusted to 7.0 with KOH). Cells were centrifuged again as described above and resuspended in 125 µl chilled buffer. Cells were then centrifuged and resuspended two more times in the same HEPES buffer as described above at a volume of 100 µl and 750 µl, respectively. Resuspended cells were then distributed into 40 µl aliquots, quickly frozen in liquid nitrogen, and stored at -80° C.

*Agrobacterium* cells were transformed with plasmids prepared as described above following the protocol described by Nagel et al. *FEMS Microbiol Letts* 67: 325-328 (1990). For each DNA construct to be transformed, 50 – 100 ng DNA (generally resuspended in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) was mixed with 40 µl of *Agrobacterium* cells. The DNA/cell mixture was then transferred to a chilled cuvette with a 2mm electrode gap and subject to a 2.5 kV charge dissipated at 25 µF and 200 µF using a Gene Pulser II apparatus (Bio-Rad). After electroporation, cells were immediately resuspended in 1.0 ml LB and allowed to recover without antibiotic selection for 2 – 4 hours at 28° C in a shaking incubator. After recovery, cells were plated onto selective medium of LB broth containing 100 µg/ml spectinomycin (Sigma) and incubated for 24-48 hours at 28° C. Single colonies were then picked and inoculated in fresh medium. The integrity of the plasmid construct was verified by PCR amplification and sequence analysis.

**Example V. Transformation of *Arabidopsis* Plants with *Agrobacterium tumefaciens* with Expression Vector**

After transformation of *Agrobacterium tumefaciens* with plasmid vectors containing the gene, single *Agrobacterium* colonies were identified, propagated, and used to transform *Arabidopsis* plants. Briefly, 500 ml cultures of LB medium containing 50 mg/l spectinomycin were inoculated with the colonies and grown at 28° C with shaking for 2 days until an absorbance ( $A_{600}$ ) of > 2.0 is reached. Cells were then harvested by centrifugation at 4,000 x g for 10 min, and resuspended in infiltration medium (1/2 X Murashige and Skoog salts (Sigma), 1 X Gamborg's B-5 vitamins (Sigma), 5.0% (w/v) sucrose (Sigma), 0.044  $\mu$ M benzylamino purine (Sigma), 200  $\mu$ l/L Silwet L-77 (Lehle Seeds) until an absorbance ( $A_{600}$ ) of 0.8 was reached.

Prior to transformation, *Arabidopsis thaliana* seeds (ecotype Columbia) were sown at a density of ~10 plants per 4" pot onto Pro-Mix BX potting medium (Hummert International) covered with fiberglass mesh (18 mm X 16 mm). Plants were grown under continuous illumination (50-75  $\mu$ E/m<sup>2</sup>/sec) at 22-23° C with 65-70% relative humidity. After about 4 weeks, primary inflorescence stems (bolts) are cut off to encourage growth of multiple secondary bolts. After flowering of the mature secondary bolts, plants were prepared for transformation by removal of all siliques and opened flowers.

The pots were then immersed upside down in the mixture of *Agrobacterium* infiltration medium as described above for 30 sec, and placed on their sides to allow draining into a 1' x 2' flat surface covered with plastic wrap. After 24 h, the plastic wrap was removed and pots are turned upright. The immersion procedure was repeated one week later, for a total of two immersions per pot. Seeds were then collected from each transformation pot and analyzed following the protocol described below.

**Example VI. Identification of *Arabidopsis* Primary Transformants**

Seeds collected from the transformation pots were sterilized essentially as follows. Seeds were dispersed into in a solution containing 0.1% (v/v) Triton X-100 (Sigma) and sterile H<sub>2</sub>O and washed by shaking the suspension for 20 min. The wash solution was then drained and replaced with fresh wash solution to wash the seeds for 20 min with shaking. After removal of the second wash solution, a solution containing 0.1% (v/v) Triton X-100 and 70% ethanol (Equistar) was added to the seeds and the suspension was shaken for 5 min. After removal of the ethanol/detergent solution, a solution containing 0.1% (v/v) Triton X-100 and 30% (v/v) bleach (Clorox) was added to the seeds, and the suspension was shaken for 10 min. After removal of the bleach/detergent solution, seeds were then washed five times in sterile distilled H<sub>2</sub>O. The seeds were stored in the last wash water at 4° C for 2 days in the dark before being plated onto antibiotic selection medium (1 X Murashige and Skoog salts (pH

adjusted to 5.7 with 1M KOH), 1 X Gamborg's B-5 vitamins, 0.9% phytagar (Life Technologies), and 50 mg/l kanamycin). Seeds were germinated under continuous illumination ( $50-75 \mu\text{E}/\text{m}^2/\text{sec}$ ) at  $22-23^\circ\text{C}$ . After 7-10 days of growth under these conditions, kanamycin resistant primary transformants ( $T_1$  generation) were visible and obtained. These seedlings were transferred first to fresh selection plates where the seedlings continued to grow for 3-5 more days, and then to soil (Pro-Mix BX potting medium). Primary transformants are self-crossed and progeny seeds ( $T_2$ ) collected.  $T_2$  progeny seeds were germinated on kanamycin as described above and kanamycin resistant seedlings were selected, transferred to soil and analyzed.

#### Example VII. Analysis of transgenic Arabidopsis plants

In a first experiment, G157 plants (ie plants expressing the G157 transgene) were grown in 12 hours light. 31 of 40 lines flowered earlier than control plants transformed with a control vector. Mean rosette leaf number of early  $T_1$  lines was  $12.4 \pm 0.8$  whereas control lines had  $27 \pm 1.2$  rosette leaves. 2 of 40  $T_1$  plants flowered at the same time as controls and 7 of 40 lines were late flowering and produced visible inflorescences 2 to 3 weeks after wild type.

In further experiments, plants were grown under conditions of 24 hours light at  $20-25^\circ\text{C}$ . Under these conditions, the non-transformed control plants produced a mean total of  $14.3 \pm 0.7$  leaves on the primary shoots prior to flower bud initiation. Flower buds were first visible on these plants at a mean of  $21.1 \pm 0.5$  days after sowing (error values represent standard error of the mean to which 95% confidence limits have been attached). For G859, 14/19  $T_1$  plants were early flowering (mean leaf total of  $6.4 \pm 0.7$ , flower buds visible at  $12.9 \pm 0.7$  days after sowing), 3/19 were wild type, and 2/19 were slightly late flowering compared to wild type (mean total of 19 leaves, flower buds visible at 27 days). RT expression studies revealed that the late flowering individuals possessed the highest levels of transgene expression. These results strongly parallel those obtained for G157. For G1842, 7/10  $T_1$  flowered early (mean total of  $7.9 \pm 0.6$  leaves, flower buds visible at  $13.9 \pm 1.0$  days), and 3/10 plants were wild type. Overexpression studies were also performed with cDNAs encoding shortened splice variants of G1842. For G1842.2 (encodes a 185 amino acid splice variant), 15/18  $T_1$  plants flowered early (mean total of  $6.9 \pm 0.9$  leaves, flower buds visible at  $14.5 \pm 0.6$  days) and 3/18 were wild type. For G1842.6 (encodes a 77 amino acid splice variant), 8/10  $T_1$  plants flowered early mean total of  $6.8 \pm 1.6$  leaves, flower buds visible at  $13.9 \pm 0.9$  days) and 2/10 were wild type. For G1842.7 (encodes a 118 amino acid splice variant) 8/10  $T_1$  plants flowered early (accurate leaf counts not made) and 2/10 were wild type. Thus, the G1842 splice variants produced comparable effects to the full-length cDNA

clone when over-expressed. For G1843, 7/11 flowered early (mean total of  $6.4 \pm 0.5$  leaves, flower buds visible at  $16.0 \pm 1.6$  days) and 2/11 had a wild type flowering time. The G1843 T1 plants, however, were dwarfed and showed retarded development of some organs. This suggests that G1843 has unpredicted toxic effects when over-expressed. For G1844, 6/10 T1 plants flowered early (mean total of  $6.8 \pm 1.7$  leaves, flower buds visible at  $14.7 \pm 1.3$  days) and 4/10 plants were wild type. Overexpression studies were also performed with a cDNA encoding a shortened splice variant of G1844. For overexpression of G1844.2 (encodes a 184 amino acid splice variant), 6/19 T1 plants flowered early (mean total of  $7.8 \pm 1.7$  leaves, flower buds visible at  $15.7 \pm 1.3$  days) and 13/19 were wild type). The over-expression data for G859, G1842, G1843, and G1844 support the hypothesis that they have a role in the control of flowering time.

RT-PCR was performed on materials from G157 plants using G157 specific primers at approximately 25 cycles. The highest levels of G157 expression were detected in late flowering individual plants or in samples from pooled seedlings that contained late flowering individuals. Plants that showed only moderate or low levels of overexpression compared to wild type were slightly early flowering or normal.

To test whether an increase in G157 could affect flowering time in late flowering ecotypes of Arabidopsis, we overexpressed G157 in the late flowering ecotypes Stockholm and Pitztal. In this experiment, 32 primary transformants from each ecotype were grown interspersed with controls under continuous light conditions. In both ecotypes, around 50% of the transformants flowered earlier than controls, and in some transformants the time to flowering was halved. As was observed with Columbia G157 plants, a minority of Pitztal and Stockholm transformants were clearly later flowering compared to controls.

A correlation between G157 transgene expression and flowering time was also observed in G157 Stockholm and Pitztal T1 plants. RT-PCR was performed with two early and two late flowering lines in each background. Again, the late flowering lines contained the higher levels of G157 expression. Thus, the factor appears to affect flowering time in a quantitative manner; a modest level of overexpression triggers early flowering, whereas a larger increase delays flowering.

In conclusion, over-expression of G157 or any of the related genes modifies flowering time in plants: a modest level of over-expression triggers early flowering, whereas a larger increase delays flowering.

Using similar or identical methodologies described in the examples above, further Arabidopsis genes were identified whose altered expression was correlated with delayed or accelerated flowering. These genes are tabulated in Table 2 with their Sequence Listing Nos., and their effects on flowering time.



Table 2. Further Arabidopsis genes for manipulating flowering time

SEQ ID Nos.	Gene	observations
23, 24	G861	early or late flowering
25, 26	G861.1	early or late flowering
29, 30	G192	late flowering
31, 32	G234	late flowering
33, 34	G361	late flowering
35, 36	G486	late flowering
37, 38	G748	late flowering
39, 40	G994	late flowering
41, 42	G1335	late flowering
43, 44	G562	late flowering
45, 46	G736	late flowering
47, 48	G1073	late flowering
49, 50	G1435	late flowering
51, 52	G180	early flowering
53, 54	G592	early flowering
55, 56	G208	early flowering

5           The vernalization response was also investigated. Late flowering vernalization responsive ecotypes and mutants have high steady state levels of *FLC* transcript, which decrease during the promotion of flowering by vernalization (Michaels and Amasino, (1999) *Plant Cell* 11:949-956; Sheldon et al., (1999) *Plant Cell* 11:445-458; Sheldon et al., (2000) *Proc. Natl. Acad. Sci.* 97: 3735-3758). In contrast to *FLC*, *G157* transcript levels show no

10 consistent correlation with the vernalization response in the late flowering Stockholm and Pitztal ecotypes. Additionally we found that over-expression of *G157* did not influence *FLC* levels. The effects of vernalization on expression of *G861*, *G859*, *G1842*, *G1843*, and *G1844* were also examined. Germinating seeds of Columbia, Pitztal, Stockholm, *constans-1*, and *fca-9* were vernalized on MS agar plates in a 4°C cold room for 8 weeks, and then transferred

15 to a continuous light growth room. Total tissues from the vernalized seedlings, and freshly sown non-vernalized controls were harvested at 9 days after the transfer. RT-PCR was performed for *FLC*, *G157*, *G859*, *G1842*, *G1843*, *G1844*, and *G861*, and actin. Compared to *FLC* and *G157*, none of the genes showed a clear consistent decline upon vernalization in the five different sample sets. However, *G1844* displayed a converse pattern of expression to

20 *FLC*: *G1844* levels consistently increased on vernalization. This is particularly significant as it directly implicates *G1844* in control of the vernalization response. Thus *G1844* likely activates flowering and has an opposing role to *FLC*.

To explore whether overexpression of G157 produces comparable effects on vernalization, batches of wild type Pitztal and Stockholm seedlings were cold treated for 6 weeks at 4°C, then grown amongst a second selection of G157 T1 Pitztal, G157 T1 Stockholm and non-vernalized wild type plants. As expected, vernalization markedly and uniformly reduced flowering time in both Pitztal and Stockholm wild type plants. Amongst the G157 Stockholm lines, the earliest flowering T1 group (8/23 lines) was indistinguishable from vernalized plants. For Pitztal, however, the early flowering T1 plants were on average marginally later than the vernalized plants. Therefore, overexpression of G157 can substantially reduce the requirement for vernalization in late flowering ecotypes.

Additionally, we observed that the late flowering of G157 lines is independent of FLC expression and does not respond to vernalization. However, the late flowering G157 plants are responsive to photoperiod. In an experiment conducted under short day conditions of 8 hours of light, we obtained a number of G157 Columbia T1 plants that flowered up to a month later than wild type controls (data not shown). To confirm that the late flowering effects caused by G157 overexpression were independent of *FLC* transcription, we tested whether late flowering G157 Columbia plants were responsive to vernalization. No significant change in flowering time was noted: in continuous light conditions, vernalized T2 plants of line 4 had a total of 31.3 +/- 1.8 leaves compared to 30.1 +/- 1.3 when non-vernalized. Control *fca* plants verified that the treatment was effective: vernalized plants flowered after only 10.3 +/- 0.9 leaves compared to more than 40 leaves for the non-vernalized controls. Thus, the late flowering phenotype caused by G157 could not be overcome by vernalization, as would be expected if the delay occurred independently of changes in *FLC* expression

#### Example IX. Identification of Homologous Sequences

Homologs from plant species other than *Arabidopsis* were identified using database sequence search tools, such as the Basic Local Alignment Search Tool (BLAST) (Altschul et al. (1990) *J. Mol. Biol.* 215:403-410; and Altschul et al. (1997) *Nucl. Acid Res.* 25: 3389-3402). The tblastx sequence analysis programs were employed using the BLOSUM-62 scoring matrix (Henikoff, S. and Henikoff, J. G. (1992) *Proc. Natl. Acad. Sci. USA* 89: 10915-10919).

The entire NCBI Genbank database was filtered for sequences from all plants except *Arabidopsis thaliana* by selecting all entries in the NCBI Genbank database associated with NCBI taxonomic ID 33090 (Viridiplantae; all plants) and excluding entries associated with taxonomic ID 3701 (*Arabidopsis thaliana*). These sequences were compared to sequences representing genes of SEQ IDs 1-56 on 9/26/2000 using the Washington University TBLASTX algorithm (version 2.0a19MP). For each gene of SEQ IDs 1-56, individual comparisons were ordered by probability score (P-value), where the score reflects the probability that a particular alignment occurred by chance. For example, a score of 3.6e-40 is  $3.6 \times 10^{-40}$ . For up to ten

species, the gene with the lowest P-value (and therefore the most likely homolog) is listed in Figure 2.

In addition to P-values, comparisons were also scored by percentage identity.

Percentage identity reflects the degree to which two segments of DNA or protein are identical over a particular length. The ranges of percent identity between the non-Arabidopsis genes shown in Figure 2 and the Arabidopsis genes in the sequence listing are: SEQ ID No. 1: 54%-67%; SEQ ID Nos. 3,5,7: 37%-47%; SEQ ID Nos. 9,11,13,15: 54%-62%; SEQ ID No. 17: 62%-71%; SEQ ID Nos. 19, 21: 50%-67%; SEQ ID Nos. 23,25: 75%-91%; SEQ ID No. 27: 46%-69%; SEQ ID No. 29: 44%-90%; SEQ ID No. 31: 57-89%; SEQ ID No. 33: 37%-79%; SEQ ID No. 35: 50%-71%; SEQ ID No. 37: 39%-63%; SEQ ID No. 39: 58%-70%; SEQ ID No. 41: 45%-73%; SEQ ID No. 43: 42%-84%; SEQ ID No. 45: 47%-81%; SEQ ID No. 47: 31%-71%; SEQ ID No. 49: 40%-67%; SEQ ID No. 51: 69%-51%; SEQ ID No. 53: 43%-86%; and SEQ ID No. 55: 79%-89%.

Arabidopsis homologs of genes in Table 2 were also identified using BLAST. These genes are found in the following Arabidopsis BAC sequences, identified by their Genbank sequence NID numbers: 2827698 (G234 homolog), 3241917 (G748 homolog), 2618604 (G994 homolog), 6598548 (G1335 homolog), 7340331 (G736 homolog), 6523051 (G1435 homolog), 6598491 (G208 homolog) and 3172156 (G208 homolog).

All references (publications and patents) are incorporated herein by reference in their entirety for all purposes.

Although the invention has been described with reference to the embodiments and examples above, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

Figure 1

SEQ ID No.	Gene	cDNA or	conserved domain
1	G157	cDNA	
2	G157	protein	2-57
3	G859	cDNA	
4	G859	protein	2-57
5	G859.1	cDNA	
6	G859.1	protein	2-57
7	G859.2	cDNA	
8	G859.2	protein	2-57
9	G1842	cDNA	
10	G1842	protein	2-57
11	G1842.2	cDNA	
12	G1842.2	protein	2-57
13	G1842.6	cDNA	
14	G1842.6	protein	2-57
15	G1842.7	cDNA	
16	G1842.7	protein	2-57
17	G1843	cDNA	
18	G1843	protein	2-57
19	G1844	cDNA	
20	G1844	protein	2-57
21	G1844.2	cDNA	
22	G1844.2	protein	2-57
23	G861	cDNA	
24	G861	protein	2-57
25	G861.1	cDNA	
26	G861.1	protein	2-57
27	G1759	cDNA	
28	G1759	protein	2-57
29	G192	cDNA	
30	G192	protein	128-185
31	G234	cDNA	
32	G234	protein	14-115
33	G361	cDNA	
34	G361	protein	43-63
35	G486	cDNA	
36	G486	protein	5-66
37	G748	cDNA	
38	G748	protein	112-140
39	G994	cDNA	
40	G994	protein	14-123
41	G1335	cDNA	
42	G1335	protein	24-43, 131-144, 185-203
43	G562	cDNA	
44	G562	protein	253-315
45	G736	cDNA	
46	G736	protein	54-111
47	G1073	cDNA	
48	G1073	protein	33-42, 78-175
49	G1435	cDNA	
50	G1435	protein	146-194
51	G180	cDNA	
52	G180	protein	118-174
53	G592	cDNA	
54	G592	protein	290-342
55	G208	cDNA	
56	G208	protein	14-116

Figure 2A

SEQ IDs	Gene Ids	Genbank NID	P-value	Species
1	G157	6530836	3.10E-22	Lycopersicon esculentum
1	G157	5606765	5.50E-14	Glycine max
1	G157	6826955	1.20E-13	Zea mays
1	G157	6536942	6.00E-13	Medicago truncatula
1	G157	8707754	1.40E-12	Hordeum vulgare
1	G157	2293891	1.40E-12	Petunia x hybrida
1	G157	19870	1.40E-12	Nicotiana tabacum
1	G157	7628118	3.70E-12	Gossypium arboreum
1	G157	5050220	3.80E-12	Gossypium hirsutum
1	G157	9414215	4.50E-12	Triticum aestivum
3,5,7	G859	6530836	1.40E-34	Lycopersicon esculentum
3,5,7	G859	5777903	4.70E-30	Malus domestica
3,5,7	G859	9367312	7.10E-30	Hordeum vulgare
3,5,7	G859	6467973	3.60E-29	Dendrobium grex Madame Thong-IN
3,5,7	G859	4204233	1.20E-28	Lolium temulentum
3,5,7	G859	939784	2.50E-28	Zea mays
3,5,7	G859	6651032	3.10E-28	Capsicum annuum
3,5,7	G859	1483227	4.60E-28	Betula pendula
3,5,7	G859	5295983	8.70E-28	Oryza sativa
3,5,7	G859	5070137	1.10E-27	Nicotiana sylvestris
9,11,13,15	G1842	6530836	5.90E-19	Lycopersicon esculentum
9,11,13,15	G1842	5606765	8.00E-15	Glycine max
9,11,13,15	G1842	6826955	1.20E-12	Zea mays
9,11,13,15	G1842	4979250	1.50E-11	Oryza sativa
9,11,13,15	G1842	6536942	1.50E-11	Medicago truncatula
9,11,13,15	G1842	7501504	4.00E-11	Gossypium arboreum
9,11,13,15	G1842	9444818	4.70E-11	Triticum aestivum
9,11,13,15	G1842	5859176	5.40E-11	Pinus taeda
9,11,13,15	G1842	5777905	6.80E-11	Malus domestica
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17	G1843	7145381	6.30E-14	Zea mays
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17	G1843	4528048	2.30E-13	Citrus unshiu
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19, 21	G1844	8707754	3.30E-13	Hordeum vulgare
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19, 21	G1844	7628118	1.10E-12	Gossypium arboreum
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19, 21	G1844	6530836	1.40E-12	Lycopersicon esculentum
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19, 21	G1844	6536942	3.50E-12	Medicago truncatula
19, 21	G1844	2252481	3.70E-12	Ceratopteris richardii
23,25	G861	5601313	8.20E-49	Lycopersicon esculentum
23,25	G861	2735763	1.50E-37	Solanum tuberosum
23,25	G861	6652755	5.40E-37	Paulownia kawakamii

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Figure 2B

SEQ IDs	Gene Ids	Genbank NID	P-value	Species
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23,25	G861	3986688	5.20E-26	Cichorium intybus
23,25	G861	7552197	2.40E-25	Sorghum bicolor
23,25	G861	5295977	4.90E-24	Oryza sativa
23,25	G861	9194959	3.60E-19	Medicago truncatula
23,25	G861	3855425	4.40E-19	Populus tremula x Populus tremuloides
23,25	G861	5606765	4.60E-16	Glycine max
27	G1759	7647685	4.10E-15	Lycopersicon esculentum
27	G1759	4979250	2.70E-14	Oryza sativa
27	G1759	8707754	6.30E-14	Hordeum vulgare
27	G1759	5777905	6.80E-14	Malus domestica
27	G1759	7626240	1.10E-13	Gossypium arboreum
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27	G1759	6918768	1.20E-13	Zea mays
27	G1759	8574456	1.30E-13	Capsicum annuum
27	G1759	8216956	1.30E-13	Cucumis sativus
27	G192	7284340	3.60E-40	Glycine max
29	G192	7779802	1.10E-39	Lotus japonicus
29	G192	9361307	9.40E-28	Triticum aestivum
29	G192	7340336	8.10E-24	Oryza sativa
29	G192	6529152	4.70E-23	Lycopersicon esculentum
29	G192	7206269	2.90E-22	Medicago truncatula
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29	G192	8706346	4.70E-13	Hordeum vulgare
29	G192	9302479	8.80E-13	Sorghum bicolor
29	G192	3326241	2.40E-12	Gossypium hirsutum
29	G192	9193243	7.50E-60	Medicago truncatula
31	G234	9264511	3.30E-57	Glycine max
31	G234	7412424	3.60E-49	Lycopersicon esculentum
31	G234	8335078	2.60E-48	Oryza sativa
31	G234	7218651	1.00E-42	Sorghum bicolor
31	G234	9364630	9.90E-40	Triticum aestivum
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31	G234	9252441	5.40E-35	Solanum tuberosum
31	G234	5860031	1.00E-33	Pinus taeda
31	G234	5050757	2.60E-33	Gossypium hirsutum
31	G234	7561045	2.30E-21	Medicago truncatula
33	G361	9307604	1.20E-17	Sorghum bicolor
33	G361	4119050	1.70E-13	Oryza sativa
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33	G361	8329902	5.30E-09	Mesembryanthemum crystallinum
33	G361	6534259	1.20E-08	Lycopersicon esculentum
33	G361	7283798	1.30E-08	Glycine max
33	G361	3854369	5.50E-08	Populus tremula x Populus tremuloides
33	G361	9365078	1.70E-07	Triticum aestivum
33	G361	5268965	0.00023	Zea mays
35	G486	6845875	3.10E-36	Glycine max
35	G486	8172030	4.20E-29	Medicago truncatula
35	G486	9416562	6.40E-29	Triticum aestivum
35	G486	5050127	4.90E-28	Gossypium hirsutum
35	G486	7628400	6.10E-28	Gossypium arboreum
35	G486	7781090	2.10E-27	Lotus japonicus

Figure 2C

SEQ IDs	Gene Ids	Genbank NID	P-value	Species
35	G486	22379	3.40E-27	Zea mays
35	G486	9441376	4.80E-27	Chlamydomonas reinhardtii
35	G486	7409616	1.30E-26	Lycopersicon esculentum
35	G486	8071558	1.40E-26	Solanum tuberosum
37	G748	853689	5.60E-87	Cucurbita maxima
37	G748	7242897	3.10E-59	Oryza sativa
37	G748	5888560	9.70E-46	Lycopersicon esculentum
37	G748	6341666	4.50E-38	Glycine max
37	G748	9190140	2.90E-35	Medicago truncatula
37	G748	7535776	4.00E-33	Sorghum bicolor
37	G748	9419494	1.70E-31	Hordeum vulgare
37	G748	9410157	8.20E-29	Triticum aestivum
37	G748	3929324	3.50E-25	Dendrobium grex Madame Thong-IN
37	G748	6020953	7.30E-21	Zea mays
39	G994	6651291	1.50E-55	Pimpinella brachycarpa
39	G994	7561750	5.60E-51	Medicago truncatula
39	G994	5268844	2.10E-50	Zea mays
39	G994	1430845	3.10E-50	Lycopersicon esculentum
39	G994	1945282	5.40E-49	Oryza sativa
39	G994	22637	1.40E-46	Physcomitrella patens
39	G994	7626566	4.40E-44	Gossypium arboreum
39	G994	2921339	4.50E-44	Gossypium hirsutum
39	G994	7590249	3.60E-43	Glycine max
39	G994	20562	6.30E-43	Petunia x hybrida
41	G1335	19742	8.40E-63	Nicotiana sylvestris
41	G1335	5398738	1.20E-59	Zea mays
41	G1335	9361467	1.40E-50	Triticum aestivum
41	G1335	8330366	1.60E-48	Mesembryanthemum crystallinum
41	G1335	8174823	7.50E-43	Hordeum vulgare
41	G1335	6696628	8.00E-42	Pinus taeda
41	G1335	7721100	1.20E-39	Lotus japonicus
41	G1335	7502173	2.60E-37	Gossypium arboreum
41	G1335	1817176	5.60E-36	Pinus radiata
41	G1335	7550978	3.30E-35	Sorghum bicolor
43	G562	1399004	6.60E-142	Brassica napus
43	G562	5381310	6.80E-53	Catharanthus roseus
43	G562	169958	3.80E-45	Glycine max
43	G562	2879779	3.60E-43	Spinacia oleracea
43	G562	7565950	2.10E-41	Medicago truncatula
43	G562	728627	4.50E-41	Nicotiana tabacum
43	G562	1155053	2.30E-40	Phaseolus vulgaris
43	G562	1498300	5.70E-40	Petroselinum crispum
43	G562	5046889	6.70E-34	Gossypium hirsutum
43	G562	8328888	2.60E-25	Mesembryanthemum crystallinum
45	G736	7409627	1.40E-37	Lycopersicon esculentum
45	G736	9197391	5.60E-32	Medicago truncatula
45	G736	9419494	4.70E-27	Hordeum vulgare
45	G736	7328718	1.30E-25	Oryza sativa
45	G736	9410157	1.80E-25	Triticum aestivum
45	G736	853689	5.20E-25	Cucurbita maxima
45	G736	7535776	6.60E-25	Sorghum bicolor
45	G736	3929324	4.70E-21	Dendrobium grex Madame Thong-IN
45	G736	2393774	9.60E-20	Zea mays

Figure 2D

SEQ IDs	Gene Ids	Genbank NID	P-value	Species
45	G736	7624398	1.10E-19	Gossypium arboreum
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47	G1073	6846994	2.50E-44	Glycine max
47	G1073	7615218	1.60E-42	Lotus japonicus
47	G1073	7333102	2.70E-34	Lycopersicon esculentum
47	G1073	9445090	3.40E-25	Triticum aestivum
47	G1073	9252370	2.20E-24	Solanum tuberosum
47	G1073	5042437	4.60E-21	Oryza sativa
47	G1073	7536402	5.30E-20	Sorghum bicolor
47	G1073	2213535	7.30E-19	Pisum sativum
47	G1073	7624850	2.10E-18	Gossypium arboreum
49	G1435	9203811	3.70E-37	Glycine max
49	G1435	9430136	4.10E-35	Lycopersicon esculentum
49	G1435	8904354	4.30E-32	Hordeum vulgare
49	G1435	5050706	3.30E-26	Gossypium hirsutum
49	G1435	7614196	6.40E-19	Lotus japonicus
49	G1435	7551484	1.00E-18	Sorghum bicolor
49	G1435	6916552	7.20E-12	Lycopersicon pennellii
49	G1435	2443007	5.50E-11	Oryza sativa
49	G1435	9255229	1.30E-10	Zea mays
49	G1435	7766737	2.80E-10	Medicago truncatula
51	G180	8468047	1.90E-35	Oryza sativa
51	G180	7559831	1.20E-24	Medicago truncatula
51	G180	5272716	9.90E-24	Lycopersicon esculentum
51	G180	9187621	3.30E-23	Solanum tuberosum
51	G180	6566312	1.30E-22	Glycine max
51	G180	9304207	1.30E-21	Sorghum bicolor
51	G180	7721184	1.30E-20	Lotus japonicus
51	G180	9444636	3.10E-19	Triticum aestivum
51	G180	3220212	5.20E-19	Gossypium hirsutum
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53	G592	5896650	1.10E-22	Lycopersicon esculentum
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53	G592	9364330	1.20E-14	Triticum aestivum
53	G592	6166282	5.40E-14	Pinus taeda
53	G592	8367093	1.60E-12	Zea mays
53	G592	9301543	6.60E-11	Sorghum bicolor
53	G592	7562632	2.80E-10	Medicago truncatula
53	G592	702652	5.80E-05	Oryza sativa
53	G592	7322923	0.094	Lycopersicon hirsutum
55	G208	437326	2.80E-65	Gossypium hirsutum
55	G208	7765706	4.40E-64	Medicago truncatula
55	G208	5269878	5.80E-64	Lycopersicon esculentum
55	G208	19054	6.90E-63	Hordeum vulgare
55	G208	2605616	1.00E-62	Oryza sativa
55	G208	7626566	3.50E-62	Gossypium arboreum
55	G208	6667606	4.10E-62	Glycine max
55	G208	517492	1.80E-60	Zea mays
55	G208	9302672	2.40E-57	Sorghum bicolor
55	G208	5860031	1.30E-54	Pinus taeda



We Claim:

- 5 1. A transgenic plant comprising a recombinant polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising at least 6 consecutive amino acids of a sequence selected from the group consisting of SEQ ID Nos. 2N, where N=1-28 but excluding SEQ ID No. 28, wherein said transgenic plant has (i) a modified flowering time compared with another plant lacking the recombinant polynucleotide or (ii) a modified vernalization requirement compared with another plant lacking the recombinant polynucleotide.
- 10 2. The transgenic plant of claim 1, wherein the nucleotide sequence encodes a polypeptide comprising a conserved domain selected from the group consisting of conserved domains of SEQ ID Nos. 2N, where N=1-28.
- 15 3. The transgenic plant of claim 1, wherein the recombinant polynucleotide further comprises a promoter operably linked to said nucleotide sequence.
- 20 4. The transgenic plant of claim 3, wherein said promoter is constitutive or inducible or tissue-active.
- 25 5. The transgenic plant of claim 1, wherein said recombinant polynucleotide encodes a polypeptide comprising a conserved domain having greater than an 84% sequence identity to a sequence selected from the group consisting of SEQ ID Nos. 2N, where N=1-28.
- 30 6. A method for altering the flowering time or vernalization requirement of a plant, said method comprising (a) transforming a plant with a recombinant polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising at least 6 consecutive amino acids of a sequence selected from the group consisting of SEQ ID Nos. 2N, where N=1-28 but excluding SEQ ID No. 28, (b) selecting said transformed plants; and (c) identifying a transformed plant having an altered flowering time.
- 35 7. The method of claim 6, wherein the nucleotide sequence encodes a polypeptide comprising a conserved domain selected from the group consisting of conserved domains of SEQ ID Nos. 2N, where N=1-28.
8. The method of claim 6, wherein the recombinant polynucleotide further comprises a promoter operably linked to said nucleotide sequence.

9. The method of claim 8, wherein said promoter is constitutive or inducible or tissue-active.

5 10. The method of claim 1, wherein said recombinant polynucleotide encodes a polypeptide comprising a conserved domain having greater than an 84% sequence identity to a sequence selected from the group consisting of SEQ ID Nos. 2N, where N=1-28.

10 11. A method for altering the flowering time or vernalization requirement of a plant, said method comprising (a) transforming the plant with a recombinant polynucleotide comprising a nucleotide sequence comprising at least 18 consecutive nucleotides of a sequence selected from the group consisting of SEQ ID Nos. 2N-1, where N= 1-28, but excluding SEQ ID No. 27; and (b) selecting said transformed plant.

15 12. The method of claim 11, wherein said recombinant polynucleotide encodes a polypeptide comprising a conserved domain having greater than an 84% sequence identity to a sequence selected from the group consisting of SEQ ID Nos. 2N, where N=1-28.

20 13. A method for altering a plant's flowering time or vernalization requirement, said method comprising (a) providing a database sequence; (b) comparing said database sequence with a polypeptide selected from SEQ ID Nos. 2N, where N= 1-28; (c) selecting a database sequence that meets selected sequence criteria; and (d) transforming said selected database sequence in the plant.

25 14. The method of claim 13, wherein said recombinant polynucleotide encodes a polypeptide comprising a conserved domain having greater than an 84% sequence identity to a sequence selected from the group consisting of SEQ ID Nos. 2N, where N=1-28.

30 15. A method for altering a plant's flowering time or vernalization requirement, said method comprising (a) providing a database sequence; (b) comparing said database sequence with a polynucleotide selected from SEQ ID Nos. 2N-1, where N= 1-28; (c) selecting a database sequence that meets selected sequence criteria; and (d) transforming said selected database sequence in the plant.

35 16. The method of claim 15, wherein said recombinant polynucleotide encodes a polypeptide comprising a conserved domain having greater than an 84% sequence identity to a sequence selected from the group consisting of SEQ ID Nos. 2N, where N=1-28.

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Arg	Ile	Met	Asn	Thr	Ala	Ser	Leu	Lys	Asn	Gln	Met	Ser	Ile	Met	Gln		
				130					135					140			
gtg	tgg	ata	ctt	taa	tttctctgga	ggaacagctc	gagactgctc	tgctccgtaac								609	
Val	Trp	Ile	Leu														
				145													
tagagctagg	aagacagaac	taatgatggg	ggaagtgaag	tcccttcaaa	aaacgcatgt											669	
caaagatcat	tgatcgttat	gaaatacatc	atgctgatga	acttaaagcc	ttagatcttg											729	
cagaaaaaat	tcggaattat	cttccacaca	aggagttact	agaaatagtc	caaagattct											789	
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MBI-0021.txt

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 1 5 10 15

Arg Gln Val Thr Phe Ser Lys Arg Arg Asn Gly Leu Ile Glu Lys Ala  
 20 25 30

Arg Gln Leu Ser Ile Leu Cys Glu Ser Ser Ile Ala Val Leu Val Val  
 35 40 45

Ser Gly Ser Gly Lys Leu Tyr Lys Ser Ala Ser Gly Asp Asn Met Ser  
 50 55 60

Lys Ile Ile Asp Arg Tyr Glu Ile His His Ala Asp Glu Leu Glu Ala  
 65 70 75 80

Leu Asp Leu Ala Glu Lys Thr Arg Asn Tyr Leu Pro Leu Lys Glu Leu  
 85 90 95

Leu Glu Ile Val Gln Arg Leu Ala Gln Arg His Phe Tyr Leu Pro Leu  
 100 105 110

Leu Leu Met Lys Asn Thr Phe Phe Phe Leu Phe Phe Trp Arg Ile Met  
 115 120 125

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Leu  
 145

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aggaagaagc c atg ggt aga aaa aaa gtc gag atc aag cga atc gag aac 170  
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 1 5 10

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 Lys Ser Ser Arg Gln Val Thr Phe Ser Lys Arg Arg Asn Gly Leu Ile  
 15 20 25

## MBI-0021.txt

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gag aaa gct cga caa ctt tca att ctc tgt gaa tct tcc atc gct gtt      266
Glu Lys Ala Arg Gln Leu Ser Ile Leu Cys Glu Ser Ser Ile Ala Val
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ctc gtc gtc tcc ggc tcc gga aaa ctc tac aag tct gcc tcc ggt gac      314
Leu Val Val Ser Gly Ser Gly Lys Leu Tyr Lys Ser Ala Ser Gly Asp
                    50                      55                      60

aac atg tca aag atc att gat cgt tac gaa ata cat cat gct gat gaa      362
Asn Met Ser Lys Ile Ile Asp Arg Tyr Glu Ile His His Ala Asp Glu
                    65                      70                      75

ctt gaa gcc tta gat ctt gca gaa aaa act cgg aat tat ctg cca ctc      410
Leu Glu Ala Leu Asp Leu Ala Glu Lys Thr Arg Asn Tyr Leu Pro Leu
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aaa gag tta cta gaa ata gtc caa agg tta gca caa aga cac ttt tat      458
Lys Glu Leu Leu Glu Ile Val Gln Arg Leu Ala Gln Arg His Phe Tyr
                    95                      100                      105

ctc cct ctt ctt ctg atg aaa aat act ttt ttt ttt ctt ttc ttt tgg      506
Leu Pro Leu Leu Leu Met Lys Asn Thr Phe Phe Phe Leu Phe Phe Trp
110                      115                      120                      125

cga att atg aat aca gca agc ttg aag aat caa atg tcg ata atg caa      554
Arg Ile Met Asn Thr Ala Ser Leu Lys Asn Gln Met Ser Ile Met Gln
                    130                      135                      140

gtg tgg ata ctt taa tttctctgga ggaacagctc gagactgctc tgtccgtaac      609
Val Trp Ile Leu
                    145

tagagctagg aagacagaac taatgatggg ggaagtgaag tcccttcaaa aaacggagaa      669

cttgctgaga gaagagaacc agactttggc tagccagggtg gggaagaaga cgtttctggt      729

tatagaaggt gacagaggaa tgtcatggga aaatggctcc ggcaacaaag tacgggagac      789

tcttcgcgtg ctcaagtaat caccatcatc aacggctgag ctttcacctt aaacttacag      849

cctgattcag aagtttttac aaatttgtaa attataaaaa gcttcataat aatctcaacc      909

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20                      25                      30

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## MBI-0021.txt

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Ser Gly Ser Gly Lys Leu Tyr Lys Ser Ala Ser Gly Asp Asn Met Ser  
50 55 60

Lys Ile Ile Asp Arg Tyr Glu Ile His His Ala Asp Glu Leu Glu Ala  
65 70 75 80

Leu Asp Leu Ala Glu Lys Thr Arg Asn Tyr Leu Pro Leu Lys Glu Leu  
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Leu Glu Ile Val Gln Arg Leu Ala Gln Arg His Phe Tyr Leu Pro Leu  
100 105 110

Leu Leu Met Lys Asn Thr Phe Phe Phe Leu Phe Phe Trp Arg Ile Met  
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Leu  
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aggatcaaatt tagggcacca gccttatcgg aggaagaagc c atg ggt aga aaa aaa 176  
Met Gly Arg Lys Lys  
1 5  
gtc gag atc aag cga atc gag aac.aaa agt agt cga caa gtc act ttc 224  
Val Glu Ile Lys Arg Ile Glu Asn Lys Ser Ser Arg Gln Val Thr Phe  
10 15 20  
tcc aaa cga cgc aat ggt ctc atc gag aaa gct cga caa ctt tca att 272  
Ser Lys Arg Arg Asn Gly Leu Ile Glu Lys Ala Arg Gln Leu Ser Ile  
25 30 35  
ctc tgt gaa tct tcc atc gct gtt ctc gtc gtc tcc ggc tcc gga aaa 320  
Leu Cys Glu Ser Ser Ile Ala Val Leu Val Val Ser Gly Ser Gly Lys  
40 45 50

## MBI-0021.txt

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ctc tac aag tct gcc tcc ggt gac aac atg tca aag atc att gat cgt      368
Leu Tyr Lys Ser Ala Ser Gly Asp Asn Met Ser Lys Ile Ile Asp Arg
55                      60                      65

tac gaa ata cat cat gct gat gaa ctt gaa gcc tta gat ctt gca gaa      416
Tyr Glu Ile His His Ala Asp Glu Leu Glu Ala Leu Asp Leu Ala Glu
70                      75                      80                      85

aaa act cgg aat tat ctg cca ctc aaa gag tta cta gaa ata gtc caa      464
Lys Thr Arg Asn Tyr Leu Pro Leu Lys Glu Leu Leu Glu Ile Val Gln
90                      95                      100

agc aag ctt gaa gaa tca aat gtc gat aat gca agt gtg gat act tta      512
Ser Lys Leu Glu Glu Ser Asn Val Asp Asn Ala Ser Val Asp Thr Leu
105                      110                      115

att tct ctg gag gaa cag ctc gag act gct ctg tcc gta act aga gct      560
Ile Ser Leu Glu Glu Gln Leu Thr Ala Leu Ser Val Thr Arg Ala
120                      125                      130

agg aag aca gaa cta atg atg ggg gaa gtg aag tcc ctt caa aaa acg      608
Arg Lys Thr Glu Leu Met Met Gly Glu Val Lys Ser Leu Gln Lys Thr
135                      140                      145

gag aac ttg ctg aga gaa gag aac cag act ttg gct agc cag gtg ggg      656
Glu Asn Leu Leu Arg Glu Glu Asn Gln Thr Leu Ala Ser Gln Val Gly
150                      155                      160                      165

aag aag acg ttt ctg gtt ata gaa ggt gac aga gga atg tca tgg gaa      704
Lys Lys Thr Phe Leu Val Ile Glu Gly Asp Arg Gly Met Ser Trp Glu
170                      175                      180

aat ggc tcc ggc aac aaa gta cgg gag act ctt ccg ctg ctc aag taa      752
Asn Gly Ser Gly Asn Lys Val Arg Glu Thr Leu Pro Leu Leu Lys
185                      190                      195

tcaccatcat caacggctga gctttcacct taaacttaca gcctgattca gaagttttta      812
caaatattgta aattataaaa agcttcataa taatctcaac ctttttatct tctcgcgcc      872
aatgtggaaa ttaaggttaa aaataaaata aaacagaagc tcatgcgaaa gaattgtaaa      932
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## MBI-0021.txt

Arg Gln Leu Ser Ile Leu Cys Glu Ser Ser Ile Ala Val Leu Val Val  
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Ser Gly Ser Gly Lys Leu Tyr Lys Ser Ala Ser Gly Asp Asn Met Ser  
 50 55 60

Lys Ile Ile Asp Arg Tyr Glu Ile His His Ala Asp Glu Leu Glu Ala  
 65 70 75 80

Leu Asp Leu Ala Glu Lys Thr Arg Asn Tyr Leu Pro Leu Lys Glu Leu  
 85 90 95

Leu Glu Ile Val Gln Ser Lys Leu Glu Glu Ser Asn Val Asp Asn Ala  
 100 105 110

Ser Val Asp Thr Leu Ile Ser Leu Glu Glu Gln Leu Glu Thr Ala Leu  
 115 120 125

Ser Val Thr Arg Ala Arg Lys Thr Glu Leu Met Met Gly Glu Val Lys  
 130 135 140

Ser Leu Gln Lys Thr Glu Asn Leu Leu Arg Glu Glu Asn Gln Thr Leu  
 145 150 155 160

Ala Ser Gln Val Gly Lys Lys Thr Phe Leu Val Ile Glu Gly Asp Arg  
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 aaagaagaag atagaaacga agaaaaaaag caaacacatt ttgggtcccc ggtgggttagg 180  
 atcaaattag ggcacaaacc ttatcggaaga aagaagcc atg gga aga aga aaa gtc 236  
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## MBI-0021.txt

Met Gly Arg Arg Lys Val  
1 5

gag atc aag cga atc gag aac aaa agc agt cga caa gtc act ttc tcc 284  
Glu Ile Lys Arg Ile Glu Asn Lys Ser Ser Arg Gln Val Thr Phe Ser  
10 15 20

aaa cga cgc aaa ggt ctc atc gaa aaa gct cga caa ctt tca att ctc 332  
Lys Arg Arg Lys Gly Leu Ile Glu Lys Ala Arg Gln Leu Ser Ile Leu  
25 30 35

tgt gaa tct tcc atc gct gtt gtc gcc gtc tcc ggt tcc gga aaa ctc 380  
Cys Glu Ser Ser Ile Ala Val Val Ala Val Ser Gly Ser Gly Lys Leu  
40 45 50

tac gac tct gcc tcc ggt gac aac atg tca aag atc att gat cgt tat 428  
Tyr Asp Ser Ala Ser Gly Asp Asn Met Ser Lys Ile Ile Asp Arg Tyr  
55 60 65 70

gaa ata cat cat gct gat gaa ctt aaa gcc tta gat ctt gca gaa aaa 476  
Glu Ile His His Ala Asp Glu Leu Lys Ala Leu Asp Leu Ala Glu Lys  
75 80 85

att cgg aat tat ctt cca cac aag gag tta cta gaa ata gtc caa agc 524  
Ile Arg Asn Tyr Leu Pro His Lys Glu Leu Leu Glu Ile Val Gln Ser  
90 95 100

aag ctt gaa gaa tca aat gtc gat aat gta agt gta gat tct cta ata 572  
Lys Leu Glu Glu Ser Asn Val Asp Asn Val Ser Val Asp Ser Leu Ile  
105 110 115

tct atg gag gaa cag ctc gag act gct ctg tca gta att aga gct aag 620  
Ser Met Glu Glu Gln Leu Glu Thr Ala Leu Ser Val Ile Arg Ala Lys  
120 125 130

aag aca gaa cta atg atg gag gat atg aag tca ctt caa gaa agg gag 668  
Lys Thr Glu Leu Met Met Glu Asp Met Lys Ser Leu Gln Glu Arg Glu  
135 140 145 150

aag ttg ctg ata gaa gag aac cag att ctg gct agc cag gtg ggg aag 716  
Lys Leu Leu Ile Glu Glu Asn Gln Ile Leu Ala Ser Gln Val Gly Lys  
155 160 165

aag acg ttt ctg gtt ata gaa ggt gac aga gga atg tca cgg gaa aat 764  
Lys Thr Phe Leu Val Ile Glu Gly Asp Arg Gly Met Ser Arg Glu Asn  
170 175 180

ggc tcc ggc aac aaa gta ccg gag act ctt tcg ctg ctc aag taa 809  
Gly Ser Gly Asn Lys Val Pro Glu Thr Leu Ser Leu Leu Lys  
185 190 195

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cgccaatgtg gaaataaagg taaaacaaaa cgaagctctt ttcttttatg cgaaagaatt 989  
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MBI-0021.txt

&lt;211&gt; 196

&lt;212&gt; PRT

&lt;213&gt; Arabidopsis thaliana

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 20 25 30

Arg Gln Leu Ser Ile Leu Cys Glu Ser Ser Ile Ala Val Val Ala Val  
 35 40 45

Ser Gly Ser Gly Lys Leu Tyr Asp Ser Ala Ser Gly Asp Asn Met Ser  
 50 55 60

Lys Ile Ile Asp Arg Tyr Glu Ile His His Ala Asp Glu Leu Lys Ala  
 65 70 75 80

Leu Asp Leu Ala Glu Lys Ile Arg Asn Tyr Leu Pro His Lys Glu Leu  
 85 90 95

Leu Glu Ile Val Gln Ser Lys Leu Glu Glu Ser Asn Val Asp Asn Val  
 100 105 110

Ser Val Asp Ser Leu Ile Ser Met Glu Glu Gln Leu Glu Thr Ala Leu  
 115 120 125

Ser Val Ile Arg Ala Lys Lys Thr Glu Leu Met Met Glu Asp Met Lys  
 130 135 140

Ser Leu Gln Glu Arg Glu Lys Leu Leu Ile Glu Glu Asn Gln Ile Leu  
 145 150 155 160

Ala Ser Gln Val Gly Lys Lys Thr Phe Leu Val Ile Glu Gly Asp Arg  
 165 170 175

Gly Met Ser Arg Glu Asn Gly Ser Gly Asn Lys Val Pro Glu Thr Leu  
 180 185 190

Ser Leu Leu Lys  
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&lt;211&gt; 880

&lt;212&gt; DNA

&lt;213&gt; Arabidopsis thaliana

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<223> G1842.2
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MBI-0021.txt

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 ttgt 880

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 35 40 45

Ser Gly Ser Gly Lys Leu Tyr Asp Ser Ala Ser Gly Asp Asn Met Ser  
 50 55 60

Lys Ile Ile Asp Arg Tyr Glu Ile His His Ala Asp Glu Leu Lys Ala  
 65 70 75 80

Leu Asp Leu Ala Glu Lys Ile Arg Asn Tyr Leu Pro His Lys Glu Leu  
 85 90 95

Leu Glu Ile Val Gln Ser Val Asp Ser Leu Ile Ser Met Glu Glu Gln  
 100 105 110

Leu Glu Thr Ala Leu Ser Val Ile Arg Ala Lys Lys Thr Glu Leu Met  
 115 120 125

Met Glu Asp Met Lys Ser Leu Gln Glu Arg Glu Lys Leu Leu Ile Glu  
 130 135 140

Glu Asn Gln Ile Leu Ala Ser Gln Val Gly Lys Lys Thr Phe Leu Val  
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 165 170 175

Val Pro Glu Thr Leu Ser Leu Leu Lys  
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&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (219)..(452)

&lt;223&gt; G1842.6

&lt;400&gt; 13

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aaagaagaag atagaaacga agaaaaaag caaacacatt ttgggtcccc ggtgggttagg 180

atcaaattag ggcacaaacc ttatcggaga aagaagcc atg gga aga aga aaa gtc 236  
Met Gly Arg Arg Lys Val  
1 5gag atc aag cga atc gag aac aaa agc agt cga caa gtc act ttc tcc 284  
Glu Ile Lys Arg Ile Glu Asn Lys Ser Ser Arg Gln Val Thr Phe Ser  
10 15 20aaa cga cgc aaa ggt ctc atc gaa aaa gct cga caa ctt tca att ctc 332  
Lys Arg Arg Lys Gly Leu Ile Glu Lys Ala Arg Gln Leu Ser Ile Leu  
25 30 35tgt gaa tct tcc atc gct gtt gtc gcc gtc tcc ggt tcc gga aaa ctc 380  
Cys Glu Ser Ser Ile Ala Val Val Ala Val Ser Gly Ser Gly Lys Leu  
40 45 50tac gac tct gcc tcc ggt gac aag atc ttg cag aaa aaa ttc gga att 428  
Tyr Asp Ser Ala Ser Gly Asp Lys Ile Leu Gln Lys Lys Phe Gly Ile  
55 60 65 70atc ttc cac aca agg agt tac tag aaatagtcca aagattctct aatatctatg 482  
Ile Phe His Thr Arg Ser Tyr  
75

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gaggatatga agtcacttca agaaaggag aagttgtcta tagaagagaa ccagattctg 602

gctagccagg tggggaagaa gacgtttctg gttatagaag gtgacagagg aatgtcacgg 662

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&lt;211&gt; 77

&lt;212&gt; PRT

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MBI-0021.txt

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 35 40 45

Ser Gly Ser Gly Lys Leu Tyr Asp Ser Ala Ser Gly Asp Lys Ile Leu  
 50 55 60

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&lt;210&gt; 15

&lt;211&gt; 876

&lt;212&gt; DNA

&lt;213&gt; Arabidopsis thaliana

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (80)..(436)

&lt;223&gt; G1842.7

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 Met Gly Arg Arg Lys Val Glu Ile Lys Arg Ile  
 1 5 10

gag aac aaa agc agt cga caa gtc act ttc tcc aaa cga cgc aaa ggt 160  
 Glu Asn Lys Ser Ser Arg Gln Val Thr Phe Ser Lys Arg Arg Lys Gly  
 15 20 25

ctc atc gaa aaa gct cga caa ctt tca att ctc tgt gaa tct tcc atc 208  
 Leu Ile Glu Lys Ala Arg Gln Leu Ser Ile Leu Cys Glu Ser Ser Ile  
 30 35 40

gct gtt gtc gcc gtc tcc ggt tcc gga aaa ctc tac gac tct gcc tcc 256  
 Ala Val Val Ala Val Ser Gly Ser Gly Lys Leu Tyr Asp Ser Ala Ser  
 45 50 55

ggt gac aac atg tca aag atc att gat cgt tat gaa ata cat cat gct 304  
 Gly Asp Asn Met Ser Lys Ile Ile Asp Arg Tyr Glu Ile His His Ala  
 60 65 70 75

gat gaa ctt aaa gcc tta gat ctt gca gaa aaa att cgg aat tat ctt 352  
 Asp Glu Leu Lys Ala Leu Asp Leu Ala Glu Lys Ile Arg Asn Tyr Leu  
 80 85 90

cca cac aag gag tta cta gaa ata gtc caa aga ttc tct aat atc tat 400  
 Pro His Lys Glu Leu Leu Glu Ile Val Gln Arg Phe Ser Asn Ile Tyr  
 95 100 105

MBI-0021.txt

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 gagaaccaga ttctggctag ccaggtgggg aagaagacgt ttctggttat agaaggtag 566  
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 35 40 45

Ser Gly Ser Gly Lys Leu Tyr Asp Ser Ala Ser Gly Asp Asn Met Ser  
 50 55 60

Lys Ile Ile Asp Arg Tyr Glu Ile His His Ala Asp Glu Leu Lys Ala  
 65 70 75 80

Leu Asp Leu Ala Glu Lys Ile Arg Asn Tyr Leu Pro His Lys Glu Leu  
 85 90 95

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<210> 17  
 <211> 818



MBI-0021.txt

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Arg Gln Leu Ser Ile Leu Cys Glu Ser Ser Val Ala Leu Ile Ile Ile  
35 40 45

Ser Ala Thr Gly Arg Leu Tyr Ser Phe Ser Ser Gly Asp Ser Met Ala  
50 55 60

Lys Ile Leu Ser Arg Tyr Glu Leu Glu Gln Ala Asp Asp Leu Lys Thr  
65 70 75 80

Leu Asp Leu Glu Glu Lys Thr Leu Asn Tyr Leu Ser His Lys Glu Leu  
85 90 95

Leu Glu Thr Ile Gln Cys Lys Ile Glu Glu Ala Lys Ser Asp Asn Val  
100 105 110

Ser Ile Asp Cys Leu Lys Ser Leu Glu Glu Gln Leu Lys Thr Ala Leu  
115 120 125

Ser Val Thr Arg Ala Arg Lys Thr Glu Leu Met Met Glu Leu Val Lys  
130 135 140

Thr His Gln Glu Lys Glu Lys Leu Leu Arg Glu Glu Asn Gln Ser Leu  
145 150 155 160

Thr Asn Gln Leu Ile Lys Met Gly Lys Met Lys Lys Ser Val Glu Ala  
165 170 175

Glu Asp Ala Arg Ala Met Ser Pro Glu Ser Ser Ser Asp Asn Lys Pro  
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MBI-0021.txt

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gag atc aaa cga att gag aac aaa agc agt aga caa gtc act ttc tgt 104  
Glu Ile Lys Arg Ile Glu Asn Lys Ser Ser Arg Gln Val Thr Phe Cys  
10 15 20

aag aga cga aat ggt ctc atg gag aaa gct cgt caa ctc tca att ctc 152  
Lys Arg Arg Asn Gly Leu Met Glu Lys Ala Arg Gln Leu Ser Ile Leu  
25 30 35

tgt gga tcc tcc gtc gct ctt ttc atc gtc tct tcc acc ggc aaa ctc 200  
Cys Gly Ser Ser Val Ala Leu Phe Ile Val Ser Ser Thr Gly Lys Leu  
40 45 50

tac aac tcc tcc tcc ggc gac agc atg gcc aag atc atc agt cgt ttt 248  
Tyr Asn Ser Ser Ser Gly Asp Ser Met Ala Lys Ile Ile Ser Arg Phe  
55 60 65 70

aaa ata caa caa gct gat gat cct gaa acc ttg gat ctt gaa gac aaa 296  
Lys Ile Gln Gln Ala Asp Asp Pro Glu Thr Leu Asp Leu Glu Asp Lys  
75 80 85

act cag gat tat ctt tca cac aag gag tta cta gaa ata gtt caa aga 344  
Thr Gln Asp Tyr Leu Ser His Lys Glu Leu Leu Glu Ile Val Gln Arg  
90 95 100

aag att gaa gaa gca aaa ggg gat aat gta agt ata gaa tct cta att 392  
Lys Ile Glu Glu Ala Lys Gly Asp Asn Val Ser Ile Glu Ser Leu Ile  
105 110 115

tcc atg gaa gag cag ctc aag agt gct ctg tct gta att aga gct agg 440  
Ser Met Glu Glu Gln Leu Lys Ser Ala Leu Ser Val Ile Arg Ala Arg  
120 125 130

aag aca gag tta ttg atg gag ctt gtg aag aac ctt cag gat aag gag 488  
Lys Thr Glu Leu Leu Met Glu Leu Val Lys Asn Leu Gln Asp Lys Glu  
135 140 145 150

aag ttg ctg aaa gaa aag aac aag gtt cta gct agc gag gtg ggg aag 536  
Lys Leu Leu Lys Glu Lys Asn Lys Val Leu Ala Ser Glu Val Gly Lys  
155 160 165

ctg aag aaa att ttg gaa aca ggg gat gaa aga gca gta atg tca ccg 584  
Page 19

MBI-0021.txt

Leu Lys Lys Ile Leu Glu Thr Gly Asp Glu Arg Ala Val Met Ser Pro  
 170 175 180

gaa aat agc tct ggc cac agc cca ccg gag act ctc ccg ctt ctc aag 632  
 Glu Asn Ser Ser Gly His Ser Pro Pro Glu Thr Leu Pro Leu Leu Lys  
 185 190 195

taa ccaccaatca tcaacggctg atttttcac atcctgattc aaaaaaggta 685

aaaaaaattc atgtgtaaaa atcataaaga agctacatgt tttaaaatcc tcttctcccc 745

ctgcatacgg ataaatttat agaccaaaaa tataatgttt tccctcaa at aagatatcga 805

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 20 25 30

Arg Gln Leu Ser Ile Leu Cys Gly Ser Ser Val Ala Leu Phe Ile Val  
 35 40 45

Ser Ser Thr Gly Lys Leu Tyr Asn Ser Ser Ser Gly Asp Ser Met Ala  
 50 55 60

Lys Ile Ile Ser Arg Phe Lys Ile Gln Gln Ala Asp Asp Pro Glu Thr  
 65 70 75 80

Leu Asp Leu Glu Asp Lys Thr Gln Asp Tyr Leu Ser His Lys Glu Leu  
 85 90 95

Leu Glu Ile Val Gln Arg Lys Ile Glu Glu Ala Lys Gly Asp Asn Val  
 100 105 110

Ser Ile Glu Ser Leu Ile Ser Met Glu Glu Gln Leu Lys Ser Ala Leu  
 115 120 125

Ser Val Ile Arg Ala Arg Lys Thr Glu Leu Leu Met Glu Leu Val Lys  
 130 135 140

Asn Leu Gln Asp Lys Glu Lys Leu Leu Lys Glu Lys Asn Lys Val Leu  
 145 150 155 160

MBI-0021.txt

Ala Ser Glu Val Gly Lys Leu Lys Lys Ile Leu Glu Thr Gly Asp Glu  
 165 170 175

Arg Ala Val Met Ser Pro Glu Asn Ser Ser Gly His Ser Pro Pro Glu  
 180 185 190

Thr Leu Pro Leu Leu Lys  
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 Arg Gln Val Thr Phe Cys Lys Arg Asn Gly Leu Met Glu Lys Ala  
 20 25 30  
 cgt caa ctc tca att ctc tgt gga tcc tcc gtc gct ctt ttc atc gtc 144  
 Arg Gln Leu Ser Ile Leu Cys Gly Ser Ser Val Ala Leu Phe Ile Val  
 35 40 45  
 tct tcc acc ggc aaa ctc tac aac tcc tcc tcc ggc gac agc atg gcc 192  
 Ser Ser Thr Gly Lys Leu Tyr Asn Ser Ser Ser Gly Asp Ser Met Ala  
 50 55 60  
 aag atc atc agt cgt ttt aaa ata caa caa gct gat gat cct gaa acc 240  
 Lys Ile Ile Ser Arg Phe Lys Ile Gln Gln Ala Asp Asp Pro Glu Thr  
 65 70 75 80  
 ttg gat ctt gaa gac aaa act cag gat tat ctt tca cac aag gag tta 288  
 Leu Asp Leu Glu Asp Lys Thr Gln Asp Tyr Leu Ser His Lys Glu Leu  
 85 90 95  
 cta gaa ata gtt caa aga aag att gaa gaa gca aaa ggg gat aat gta 336  
 Leu Glu Ile Val Gln Arg Lys Ile Glu Glu Ala Lys Gly Asp Asn Val  
 100 105 110  
 agt ata gaa tct cta att tcc atg gaa gag cag ctc aag agt gct ctg 384  
 Ser Ile Glu Ser Leu Ile Ser Met Glu Glu Gln Leu Lys Ser Ala Leu  
 115 120 125  
 tct gta att aga gct agg aag aca gag tta ttg atg gag ctt gtg aag 432  
 Ser Val Ile Arg Ala Arg Lys Thr Glu Leu Leu Met Glu Leu Val Lys  
 130 135 140  
 aac ctt cag gat aag gtg ggg aag ctg aag aaa att ttg gaa aca ggg 480  
 Asn Leu Gln Asp Lys Val Gly Lys Leu Lys Lys Ile Leu Glu Thr Gly  
 145 150 155 160

## MBI-0021.txt

gat gaa aga gca gta atg tca ccg gaa aat agc tct ggc cac agc cca 528  
 Asp Glu Arg Ala Val Met Ser Pro Glu Asn Ser Ser Gly His Ser Pro  
                   165                  170                  175

ccg gag act ctc ccg ctt ctc aag taa ccaccaatca tcaacggctg 575  
 Pro Glu Thr Leu Pro Leu Leu Lys  
                   180

atttttcatc atcctgattc aaaaaaggta aaaaaaattc atgtgtaaaa atcataaaga 635

agctacatgt tttaaaatcc tcttctcccc ctgcatacgg ataaatttat agaccaaaaa 695

tataatgttt tccctcaaat aagatatoga cctttgtgtt accttggaag acaggatc 753

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                   20                  25                  30

Arg Gln Leu Ser Ile Leu Cys Gly Ser Ser Val Ala Leu Phe Ile Val  
                   35                  40                  45

Ser Ser Thr Gly Lys Leu Tyr Asn Ser Ser Ser Gly Asp Ser Met Ala  
   50                  55                  60

Lys Ile Ile Ser Arg Phe Lys Ile Gln Gln Ala Asp Asp Pro Glu Thr  
   65                  70                  75                  80

Leu Asp Leu Glu Asp Lys Thr Gln Asp Tyr Leu Ser His Lys Glu Leu  
                   85                  90                  95

Leu Glu Ile Val Gln Arg Lys Ile Glu Glu Ala Lys Gly Asp Asn Val  
                   100                  105                  110

Ser Ile Glu Ser Leu Ile Ser Met Glu Glu Gln Leu Lys Ser Ala Leu  
                   115                  120                  125

Ser Val Ile Arg Ala Arg Lys Thr Glu Leu Leu Met Glu Leu Val Lys  
                   130                  135                  140

Asn Leu Gln Asp Lys Val Gly Lys Leu Lys Lys Ile Leu Glu Thr Gly  
   145                  150                  155                  160



MBI-0021.txt

Asp Glu Arg Ala Val Met Ser Pro Glu Asn Ser Ser Gly His Ser Pro  
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Pro Glu Thr Leu Pro Leu Leu Lys  
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 tctctctctt gcttctaggg tttttttgtt cgttgtg atg gcg aga gaa aag att 175  
 Met Ala Arg Glu Lys Ile  
 1 5  
 cag atc agg aag atc gac aac gca acg gcg aga caa gtg acg ttt tcg 223  
 Gln Ile Arg Lys Ile Asp Asn Ala Thr Ala Arg Gln Val Thr Phe Ser  
 10 15 20  
 aaa cga aga aga ggg ctt ttc aag gct gaa gaa ctc tcc gtt ctc 271  
 Lys Arg Arg Arg Gly Leu Phe Lys Lys Ala Glu Glu Ser Val Leu  
 25 30 35  
 tgc gac gcc gat gtc gct ctc atc atc ttc tct tcc acc gga aaa ctg 319  
 Cys Asp Ala Asp Val Ala Leu Ile Ile Phe Ser Ser Thr Gly Lys Leu  
 40 45 50  
 ttc gag ttc tgt agc tcc agc atg aag gaa gtc cta gag agg cat aac 367  
 Phe Glu Phe Cys Ser Ser Met Lys Glu Val Leu Glu Arg His Asn  
 55 60 65 70  
 ttg cag tca aag aac ttg gag aag ctt gat cag cca tct ctt gag tta 415  
 Leu Gln Ser Lys Asn Leu Glu Lys Leu Asp Gln Pro Ser Leu Glu Leu  
 75 80 85  
 cag ctg gtt gag aac agt gat cac gcc cga atg agt aaa gaa att gcg 463  
 Gln Leu Val Glu Asn Ser Asp His Ala Arg Met Ser Lys Glu Ile Ala  
 90 95 100  
 gac aag agc cac cga cta agg caa atg aga gga gag gaa ctt caa gga 511  
 Asp Lys Ser His Arg Leu Arg Gln Met Arg Gly Glu Glu Leu Gln Gly  
 105 110 115  
 ctt gac att gaa gag ctt cag cag cta gag aag gcc ctt gaa act ggt 559  
 Leu Asp Ile Glu Glu Leu Gln Gln Leu Glu Lys Ala Leu Glu Thr Gly  
 120 125 130  
 ttg acg cgt gtg att gaa aca aag agt gac aag att atg agt gag atc 607  
 Leu Thr Arg Val Ile Glu Thr Lys Ser Asp Lys Ile Met Ser Glu Ile  
 135 140 145 150

## MBI-0021.txt

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agc gaa ctt cag aaa aag gga atg caa ttg atg gat gag aac aag cgg      655
Ser Glu Leu Gln Lys Lys Gly Met Gln Leu Met Asp Glu Asn Lys Arg
      155                                160                                165

ttg agg cag caa gga acg caa cta acg gaa gag aac gag cga ctt ggc      703
Leu Arg Gln Gln Gly Thr Gln Leu Thr Glu Glu Asn Glu Arg Leu Gly
      170                                175                                180

atg caa ata tgt aac aat gtg cat gca cac ggt ggt gct gaa tcg gag      751
Met Gln Ile Cys Asn Asn Val His Ala His Gly Gly Ala Glu Ser Glu
      185                                190                                195

aac gct gct gtg tac gag gaa gga cag tcg tcg gag tct att act aac      799
Asn Ala Ala Val Tyr Glu Glu Gly Gln Ser Ser Glu Ser Ile Thr Asn
      200                                205                                210

gcc gga aac tct acc gga gcg cct gtt gac tcc gag agc tcc gac act      847
Ala Gly Asn Ser Thr Gly Ala Pro Val Asp Ser Glu Ser Ser Asp Thr
      215                                220                                225                                230

tcc ctt agg ctc ggc tta ccg tat ggt ggt tag agatggaaca attcaaagaa      900
Ser Leu Arg Leu Gly Leu Pro Tyr Gly Gly
      235                                240

gttgatggag tgaggagagt aatgtaaactc tttttaactc ggtagtaaca agagacaatg      960
tctaagtagt gaattctcaa atgtttgtgt aagtttctgc ctatggaaga ggctttcatt      1020
tttatgattt tcaactatgta tgatctctct tcaactgcatt tctggttagt aacggcttgt      1080
caccgataaa ctttctcgtt atggaaagtt agaataaaaa aaaaaaaaaa aaaa      1134

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Met Ala Arg Glu Lys Ile Gln Ile Arg Lys Ile Asp Asn Ala Thr Ala
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Arg Gln Val Thr Phe Ser Lys Arg Arg Arg Gly Leu Phe Lys Lys Ala
      20          25          30

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Glu Glu Leu Ser Val Leu Cys Asp Ala Asp Val Ala Leu Ile Ile Phe
      35          40          45

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Ser Ser Thr Gly Lys Leu Phe Glu Phe Cys Ser Ser Ser Met Lys Glu
      50          55          60

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Val Leu Glu Arg His Asn Leu Gln Ser Lys Asn Leu Glu Lys Leu Asp
      65          70          75          80

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Gln Pro Ser Leu Glu Leu Gln Leu Val Glu Asn Ser Asp His Ala Arg
      85          90          95

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MBI-0021.txt

Met Ser Lys Glu Ile Ala Asp Lys Ser His Arg Leu Arg Gln Met Arg  
 100 105 110

Gly Glu Glu Leu Gln Gly Leu Asp Ile Glu Glu Leu Gln Gln Leu Glu  
 115 120 125

Lys Ala Leu Glu Thr Gly Leu Thr Arg Val Ile Glu Thr Lys Ser Asp  
 130 135 140

Lys Ile Met Ser Glu Ile Ser Glu Leu Gln Lys Lys Gly Met Gln Leu  
 145 150 155 160

Met Asp Glu Asn Lys Arg Leu Arg Gln Gln Gly Thr Gln Leu Thr Glu  
 165 170 175

Glu Asn Glu Arg Leu Gly Met Gln Ile Cys Asn Asn Val His Ala His  
 180 185 190

Gly Gly Ala Glu Ser Glu Asn Ala Ala Val Tyr Glu Glu Gly Gln Ser  
 195 200 205

Ser Glu Ser Ile Thr Asn Ala Gly Asn Ser Thr Gly Ala Pro Val Asp  
 210 215 220

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 gttctcgaaa gatccattaa aatcaaaacc taagctctct ctcttgcttc taggggtttt 180  
 ttgttcggtg tg atg gcg aga gaa aag att cag atc agg aag atc gac aac 231  
 Met Ala Arg Glu Lys Ile Gln Ile Arg Lys Ile Asp Asn  
 1 5 10  
 gca acg gcg aga caa gtg acg ttt tct aaa cga aga aga ggg ctt ttc 279  
 Ala Thr Ala Arg Gln Val Thr Phe Ser Lys Arg Arg Arg Gly Leu Phe  
 15 20 25

## MBI-0021.txt

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atc atc ttc tct tcc acc gga aaa ctg ttc gag ttc tgt agc tcc agc 375  
Ile Ile Phe Ser Ser Thr Gly Lys Leu Phe Glu Phe Cys Ser Ser Ser  
50 55 60  
atg aag gaa gtc cta gag agg cat aac ttg cag tca aag aac ttg gag 423  
Met Lys Glu Val Leu Glu Arg His Asn Leu Gln Ser Lys Asn Leu Glu  
65 70 75  
aag ctt gat cag cca tct ctt gag tta cag ctg gtt gag aac agt gat 471  
Lys Leu Asp Gln Pro Ser Leu Glu Leu Gln Leu Val Glu Asn Ser Asp  
80 85 90  
cac gcc cga atg agt aaa gaa att gcg gac aag agc cac cga cta agg 519  
His Ala Arg Met Ser Lys Glu Ile Ala Asp Lys Ser His Arg Leu Arg  
95 100 105  
caa atg aga gga gag gaa ctt caa gga ctt gac att gaa gag ctt cag 567  
Gln Met Arg Gly Glu Glu Leu Gln Gly Leu Asp Ile Glu Glu Leu Gln  
110 115 120 125  
cag cta gag aag gcc ctt gaa act ggt ttg acg cgt gtg att gaa aca 615  
Gln Leu Glu Lys Ala Leu Glu Thr Gly Leu Thr Arg Val Ile Glu Thr  
130 135 140  
aag agt gac aag att atg agt gag atc agc gaa ctt cag aaa aag gga 663  
Lys Ser Asp Lys Ile Met Ser Glu Ile Ser Glu Leu Gln Lys Lys Gly  
145 150 155  
atg caa ttg atg gat gag aac aag cgg ttg agg cag caa gta tgt gtc 711  
Met Gln Leu Met Asp Glu Asn Lys Arg Leu Arg Gln Gln Val Cys Val  
160 165 170  
tta ccc tct ctg ttg ata aca aat ccc ttt ctt ttg tct acc att aac 759  
Leu Pro Ser Leu Leu Ile Thr Asn Pro Phe Leu Leu Ser Thr Ile Asn  
175 180 185  
gta cac act cct aaa ttt aat ccc cag ttg tct aca aca cat atg ttt 807  
Val His Thr Pro Lys Phe Asn Pro Gln Leu Ser Thr Thr His Met Phe  
190 195 200 205  
gat cat act gtg aga taa atgaataaac caagtatat agcgcgattt 855  
Asp His Thr Val Arg  
210  
aaaaatgtct ttaaaactaa aggtaaccat gtagctagtt agtctctagg gtcctagagg 915  
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## MBI-0021.txt

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 20 25 30

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 35 40 45

Ser Ser Thr Gly Lys Leu Phe Glu Phe Cys Ser Ser Ser Met Lys Glu  
 50 55 60

Val Leu Glu Arg His Asn Leu Gln Ser Lys Asn Leu Glu Lys Leu Asp  
 65 70 75 80

Gln Pro Ser Leu Glu Leu Gln Leu Val Glu Asn Ser Asp His Ala Arg  
 85 90 95

Met Ser Lys Glu Ile Ala Asp Lys Ser His Arg Leu Arg Gln Met Arg  
 100 105 110

Gly Glu Glu Leu Gln Gly Leu Asp Ile Glu Glu Leu Gln Gln Leu Glu  
 115 120 125

Lys Ala Leu Glu Thr Gly Leu Thr Arg Val Ile Glu Thr Lys Ser Asp  
 130 135 140

Lys Ile Met Ser Glu Ile Ser Glu Leu Gln Lys Lys Gly Met Gln Leu  
 145 150 155 160

Met Asp Glu Asn Lys Arg Leu Arg Gln Gln Val Cys Val Leu Pro Ser  
 165 170 175

Leu Leu Ile Thr Asn Pro Phe Leu Leu Ser Thr Ile Asn Val His Thr  
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MBI-0021.txt

180

185

190

Pro Lys Phe Asn Pro Gln Leu Ser Thr Thr His Met Phe Asp His Thr  
 195 200 205

Val Arg  
 210

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 Met Gly Arg  
 1  
 aaa aaa cta gaa atc aag cga att gag aac aaa agt agc cga caa gtc 166  
 Lys Lys Leu Glu Ile Lys Arg Ile Glu Asn Lys Ser Ser Arg Gln Val  
 5 10 15  
 acc ttc tcc aaa cgt cgc aac ggt ctc atc gag aaa gct cgt cag ctt 214  
 Thr Phe Ser Lys Arg Arg Asn Gly Leu Ile Glu Lys Ala Arg Gln Leu  
 20 25 30 35  
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 Ser Val Leu Cys Asp Ala Ser Val Ala Leu Leu Val Val Ser Ala Ser  
 40 45 50  
 ggc aag ctc tac agc ttc tcc tcc ggc gat aac ctg gtc aag atc ctt 310  
 Gly Lys Leu Tyr Ser Phe Ser Ser Gly Asp Asn Leu Val Lys Ile Leu  
 55 60 65  
 gat cga tat ggg aaa cag cat gct gat gat ctt aaa gcc ttg gat cat 358  
 Asp Arg Tyr Gly Lys Gln His Ala Asp Asp Leu Lys Ala Leu Asp His  
 70 75 80  
 cag tca aaa gct ctg aac tat ggt tca cac tat gag cta ctt gaa ctt 406  
 Gln Ser Lys Ala Leu Asn Tyr Gly Ser His Tyr Glu Leu Leu Glu Leu  
 85 90 95  
 gtg gat agc aag ctt gtg gga tca aat gtc aaa aat gtg agt atc gat 454  
 Val Asp Ser Lys Leu Val Gly Ser Asn Val Lys Asn Val Ser Ile Asp  
 100 105 110 115  
 gct ctt gtt caa ctg gag gaa cac ctt gag act gcc ctc tcc gtg act 502  
 Ala Leu Val Gln Leu Glu Glu His Leu Glu Thr Ala Leu Ser Val Thr  
 120 125 130  
 aga gcc aag aag acc gaa ctc atg ttg aag ctt gtt gag aat ctt aaa 550  
 Arg Ala Lys Lys Thr Glu Leu Met Leu Lys Leu Val Glu Asn Leu Lys

MBI-0021.txt

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gaa aag gag aaa atg tctg aaa gaa gag aac cag gtt ttg gct agc cag      598
Glu Lys Glu Lys Met Leu Lys Glu Glu Asn Gln Val Leu Ala Ser Gln
150          155          160

atg gag aat aat cat cat gtg gga gca gaa gct gag atg gag atg tca      646
Met Glu Asn Asn His His Val Gly Ala Glu Ala Glu Met Glu Met Ser
165          170          175

cct gct gga caa atc tcc gac aat ctt ccg gtg act ctc cca cta ctt      694
Pro Ala Gly Gln Ile Ser Asp Asn Leu Pro Val Thr Leu Pro Leu Leu
180          185          190          195

aat tag ccaccttaaa tcggcgggttg aaatcaaaat ccaaaacata tataattatg      750
Asn

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taataactctc tctttggcca agagactttg tgtgtgatac ttaagtagac ggaactaagt      870
caataactatc tgttttaaga caaaagggttg atgaactttg taccttattc gtgtgagaaa      930
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Met Gly Arg Lys Lys Leu Glu Ile Lys Arg Ile Glu Asn Lys Ser Ser
1          5          10          15

Arg Gln Val Thr Phe Ser Lys Arg Arg Asn Gly Leu Ile Glu Lys Ala
20          25          30

Arg Gln Leu Ser Val Leu Cys Asp Ala Ser Val Ala Leu Leu Val Val
35          40          45

Ser Ala Ser Gly Lys Leu Tyr Ser Phe Ser Ser Gly Asp Asn Leu Val
50          55          60

Lys Ile Leu Asp Arg Tyr Gly Lys Gln His Ala Asp Asp Leu Lys Ala
65          70          75          80

Leu Asp His Gln Ser Lys Ala Leu Asn Tyr Gly Ser His Tyr Glu Leu
85          90          95

Leu Glu Leu Val Asp Ser Lys Leu Val Gly Ser Asn Val Lys Asn Val
100          105          110

Ser Ile Asp Ala Leu Val Gln Leu Glu Glu His Leu Glu Thr Ala Leu

```

MBI-0021.txt

115

120

125

Ser Val Thr Arg Ala Lys Lys Thr Glu Leu Met Leu Lys Leu Val Glu  
 130 135 140

Asn Leu Lys Glu Lys Glu Lys Met Leu Lys Glu Glu Asn Gln Val Leu  
 145 150 155 160

Ala Ser Gln Met Glu Asn Asn His His Val Gly Ala Glu Ala Glu Met  
 165 170 175

Glu Met Ser Pro Ala Gly Gln Ile Ser Asp Asn Leu Pro Val Thr Leu  
 180 185 190

Pro Leu Leu Asn  
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 ca atg gcc gac gat tgg gat ctc cac gcc gta gtc aga ggc tgc tca 107  
 Met Ala Asp Asp Trp Asp Leu His Ala Val Val Arg Gly Cys Ser  
 1 5 10 15  
 gcc gta agc tca tca gct act acc acc gta tat tcc ccc ggc gtt tca 155  
 Ala Val Ser Ser Ser Ala Thr Thr Thr Val Tyr Ser Pro Gly Val Ser  
 20 25 30  
 tct cac aca aac cct ata ttc acc gtc gga cga caa agt aat gcc gtc 203  
 Ser His Thr Asn Pro Ile Phe Thr Val Gly Arg Gln Ser Asn Ala Val  
 35 40 45  
 tcc ttc gga gag att cga gat ctc tac aca ccg ttc aca caa gaa tct 251  
 Ser Phe Gly Glu Ile Arg Asp Leu Tyr Thr Pro Phe Thr Gln Glu Ser  
 50 55 60  
 gtc gtc tct tcg ttt tct tgt ata aac tac cca gaa gaa cct aga aag 299  
 Val Val Ser Ser Phe Ser Cys Ile Asn Tyr Pro Glu Glu Pro Arg Lys  
 65 70 75  
 cca cag aac cag aaa cgt cct ctt tct ctc tct gct tct tcc ggt agc 347  
 Pro Gln Asn Gln Lys Arg Pro Leu Ser Leu Ser Ala Ser Ser Gly Ser  
 80 85 90 95  
 gtc act agc aaa ccc agt ggc tcc aat acc tct aga tct aaa aga aga 395  
 Val Thr Ser Lys Pro Ser Gly Ser Asn Thr Ser Arg Ser Lys Arg Arg



MBI-0021.txt

100	105	110	
aag ata cag cat aag aaa gtg tgc cat gta gca gca gaa gct tta aac	Lys Ile Gln His Lys Lys Val Cys His Val Ala Ala Glu Ala Leu Asn		443
	115	120	125
tcc gat gtc tgg gca tgg cga aag tac gga cag aaa ccc atc aaa ggt	Ser Asp Val Trp Ala Trp Arg Lys Tyr Gly Gln Lys Pro Ile Lys Gly		491
	130	135	140
tca cca tat cca aga gga tac tac aga tgt agt aca tca aaa ggt tgt	Ser Pro Tyr Pro Arg Gly Tyr Tyr Arg Cys Ser Thr Ser Lys Gly Cys		539
	145	150	155
tta gcc cgt aaa caa gtg gag cga aat aga tcc gac ccg aag atg ttt	Leu Ala Arg Lys Gln Val Glu Arg Asn Arg Ser Asp Pro Lys Met Phe		587
	160	165	170
atc gtc act tac acg gcg gag cat aat cat cca gct ccg aca cac cgt	Ile Val Thr Tyr Thr Ala Glu His Asn His Pro Ala Pro Thr His Arg		635
	180	185	190
aat tct ctc gcc gga agc aca cgt cag aaa cca tcc gat caa cag acg	Asn Ser Leu Ala Gly Ser Thr Arg Gln Lys Pro Ser Asp Gln Gln Thr		683
	195	200	205
agt aaa tct ccg acg acc act att gct act tat tca tcc tct ccg gtg	Ser Lys Ser Pro Thr Thr Thr Ile Ala Thr Tyr Ser Ser Ser Pro Val		731
	210	215	220
act tca gcc gac gaa ttt gtt ttg cct gtt gag gat cat cta gcg gtg	Thr Ser Ala Asp Glu Phe Val Leu Pro Val Glu Asp His Leu Ala Val		779
	225	230	235
gga gat ctt gac gga gaa gaa gat ctg tta tct ttg tcc gat acg gtg	Gly Asp Leu Asp Gly Glu Glu Asp Leu Leu Ser Leu Ser Asp Thr Val		827
	240	245	250
gtt agc gat gat ttc ttc gat ggg tta gag gaa ttc gca gcc gga gat	Val Ser Asp Asp Phe Phe Asp Gly Leu Glu Glu Phe Ala Ala Gly Asp		875
	260	265	270
agc ttt tcc ggg aac tcc gct ccg gcg agt ttt gat ctc tct tgg gtt	Ser Phe Ser Gly Asn Ser Ala Pro Ala Ser Phe Asp Leu Ser Trp Val		923
	275	280	285
gtg aac agt gcc gcc act acc acc gga gga ata tga ttagattacg	Val Asn Ser Ala Ala Thr Thr Gly Gly Ile		969
	290	295	
acggcttaga atactcttat taggacagat ttataggatt aaggaattat tctcggagca			1029
tatgtaaaaa taggataaaa gaaaatgttc tttgttactt tttttcgggt tttcttccta			1089
ttgtttctaa acatcttaga aaaaatttaa ttgtatatcc cttaagctcg atacatcttg			1149
ttttaaaaaa aaaaaaaaaa aa			1171

&lt;210&gt; 30

&lt;211&gt; 298

&lt;212&gt; PRT

&lt;213&gt; Arabidopsis thaliana

MBI-0021.txt

&lt;400&gt; 30

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Met Ala Asp Asp Trp Asp Leu His Ala Val Val Arg Gly Cys Ser Ala
1      5      10      15

Val Ser Ser Ser Ala Thr Thr Thr Val Tyr Ser Pro Gly Val Ser Ser
20      25      30

His Thr Asn Pro Ile Phe Thr Val Gly Arg Gln Ser Asn Ala Val Ser
35      40      45

Phe Gly Glu Ile Arg Asp Leu Tyr Thr Pro Phe Thr Gln Glu Ser Val
50      55      60

Val Ser Ser Phe Ser Cys Ile Asn Tyr Pro Glu Glu Pro Arg Lys Pro
65      70      75      80

Gln Asn Gln Lys Arg Pro Leu Ser Leu Ser Ala Ser Ser Gly Ser Val
85      90      95

Thr Ser Lys Pro Ser Gly Ser Asn Thr Ser Arg Ser Lys Arg Arg Lys
100     105     110

Ile Gln His Lys Lys Val Cys His Val Ala Ala Glu Ala Leu Asn Ser
115     120     125

Asp Val Trp Ala Trp Arg Lys Tyr Gly Gln Lys Pro Ile Lys Gly Ser
130     135     140

Pro Tyr Pro Arg Gly Tyr Tyr Arg Cys Ser Thr Ser Lys Gly Cys Leu
145     150     155     160

Ala Arg Lys Gln Val Glu Arg Asn Arg Ser Asp Pro Lys Met Phe Ile
165     170     175

Val Thr Tyr Thr Ala Glu His Asn His Pro Ala Pro Thr His Arg Asn
180     185     190

Ser Leu Ala Gly Ser Thr Arg Gln Lys Pro Ser Asp Gln Gln Thr Ser
195     200     205

Lys Ser Pro Thr Thr Thr Ile Ala Thr Tyr Ser Ser Ser Pro Val Thr
210     215     220

Ser Ala Asp Glu Phe Val Leu Pro Val Glu Asp His Leu Ala Val Gly
225     230     235     240

```

## MBI-0021.txt

Asp Leu Asp Gly Glu Glu Asp Leu Leu Ser Leu Ser Asp Thr Val Val  
 245 250 255

Ser Asp Asp Phe Phe Asp Gly Leu Glu Glu Phe Ala Ala Gly Asp Ser  
 260 265 270

Phe Ser Gly Asn Ser Ala Pro Ala Ser Phe Asp Leu Ser Trp Val Val  
 275 280 285

Asn Ser Ala Ala Thr Thr Thr Gly Gly Ile  
 290 295

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tatcaagaac aagactaaga acaagacttc actaggagta caagt atg gga aga gca 117  
 Met. Gly Arg Ala  
 1

ccg tgt tgt gac aaa gca aac gtg aag aaa ggg cct tgg tct cct gag 165  
 Pro Cys Cys Asp Lys Ala Asn Val Lys Lys Gly Pro Trp Ser Pro Glu  
 5 10 15 20

gaa gat gca aaa ctc aaa tct tac att gaa aat agt ggc acc gga ggc 213  
 Glu Asp Ala Lys Leu Lys Ser Tyr Ile Glu Asn Ser Gly Thr Gly Gly  
 25 30 35

aat tgg atc gct ttg cct caa aag att ggt tta aag aga tgt gga aag 261  
 Asn Trp Ile Ala Leu Pro Gln Lys Ile Gly Leu Lys Arg Cys Gly Lys  
 40 45 50

agt tgc agg ctg agg tgg ctt aac tat ctt aga cca aac atc aaa cat 309  
 Ser Cys Arg Leu Arg Trp Leu Asn Tyr Leu Arg Pro Asn Ile Lys His  
 55 60 65

ggt ggc ttc tct gag gaa gaa gaa aac atc att tgt agc ctt tac ctt 357  
 Gly Gly Phe Ser Glu Glu Glu Glu Asn Ile Ile Cys Ser Leu Tyr Leu  
 70 75 80

aca att ggt agc agg tgg tct ata atc gct gct caa ttg ccg gga cga 405  
 Thr Ile Gly Ser Arg Trp Ser Ile Ile Ala Ala Gln Leu Pro Gly Arg  
 85 90 95 100

aca gac aac gat ata aaa aac tat tgg aac acg agg ctc aag aag aaa 453  
 Thr Asp Asn Asp Ile Lys Asn Tyr Trp Asn Thr Arg Leu Lys Lys Lys  
 105 110 115

ctc att aac aaa caa cgc aag gag ctt caa gaa gct tgt atg gag cag 501  
 Page 33

MBI-0021.txt

Leu	Ile	Asn	Lys	Gln	Arg	Lys	Glu	Leu	Gln	Glu	Ala	Cys	Met	Glu	Gln			
			120					125					130					
caa	gag	atg	atg	gtg	atg	atg	aag	aga	caa	cac	caa	caa	caa	caa	atc		549	
Gln	Glu	Met	Met	Val	Met	Met	Lys	Arg	Gln	His	Gln	Gln	Gln	Gln	Ile			
		135					140					145						
caa	act	tct	ttt	atg	atg	aga	caa	gac	caa	aca	atg	ttc	aca	tgg	cca		597	
Gln	Thr	Ser	Phe	Met	Met	Arg	Gln	Asp	Gln	Thr	Met	Phe	Thr	Trp	Pro			
	150					155					160							
cta	cat	cat	cat	aat	gtt	caa	gtt	cca	gct	ctt	ttc	aga	atc	aaa	cca		645	
Leu	His	His	His	Asn	Val	Gln	Val	Pro	Ala	Leu	Phe	Arg	Ile	Lys	Pro			
	165				170					175					180			
act	cgt	ttt	gcg	acc	aag	aag	atg	tta	agc	cag	tgc	tca	tca	aga	aca		693	
Thr	Arg	Phe	Ala	Thr	Lys	Lys	Met	Leu	Ser	Gln	Cys	Ser	Ser	Ser	Thr			
				185					190					195				
tgg	tca	aga	tcg	aag	atc	aag	aac	tgg	aga	aaa	caa	acc	tca	tca	tca		741	
Trp	Ser	Arg	Ser	Lys	Ile	Lys	Asn	Trp	Arg	Lys	Gln	Thr	Ser	Ser	Ser			
			200					205					210					
tca	aga	ttc	aat	gac	aac	gct	ttt	gat	cat	ctc	tct	ttc	tct	caa	ctc		789	
Ser	Arg	Phe	Asn	Asp	Asn	Ala	Phe	Asp	His	Leu	Ser	Phe	Ser	Gln	Leu			
		215				220						225						
ttg	tta	gat	cct	aat	cat	aac	cac	tta	gga	tca	gga	gag	ggt	ttc	tcc		837	
Leu	Leu	Asp	Pro	Asn	His	Asn	His	Leu	Gly	Ser	Gly	Glu	Gly	Phe	Ser			
	230					235					240							
atg	aac	tct	atc	ttg	agc	gcc	aac	aca	aac	tct	cca	ttg	ctt	aac	aca		885	
Met	Asn	Ser	Ile	Leu	Ser	Ala	Asn	Thr	Asn	Ser	Pro	Leu	Leu	Asn	Thr			
	245				250					255					260			
agt	aat	gat	aat	cag	tgg	ttc	ggg	aat	ttc	cag	gcc	gaa	acc	gta	aac		933	
Ser	Asn	Asp	Asn	Gln	Trp	Phe	Gly	Asn	Phe	Gln	Ala	Glu	Thr	Val	Asn			
				265				270						275				
ttg	ttc	tca	gga	gcc	tcc	aca	agt	act	tcg	gca	gat	caa	agc	act	ata		981	
Leu	Phe	Ser	Gly	Ala	Ser	Thr	Ser	Thr	Ser	Ala	Asp	Gln	Ser	Thr	Ile			
			280					285					290					
agt	tgg	gaa	gac	ata	agc	tct	ctt	gtt	tat	tct	gat	tca	aag	caa	ttt		1029	
Ser	Trp	Glu	Asp	Ile	Ser	Ser	Leu	Val	Tyr	Ser	Asp	Ser	Lys	Gln	Phe			
		295					300					305						
ttt	taattataat	aatatattat	tcttaagatg	aaacgtacat	cattattatt												1082	
Phe																		
aattgggggt	acgtaacgta	tatatggaat	aacgatctag	tttggttaaa	tttaaaa												1139	
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<211>	309																	
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Met	Gly	Arg	Ala	Pro	Cys	Cys	Asp	Lys	Ala	Asn	Val	Lys	Lys	Gly	Pro			
1				5					10					15				

MBI-0021.txt

Trp Ser Pro Glu Glu Asp Ala Lys Leu Lys Ser Tyr Ile Glu Asn Ser  
 20 25 30  
 Gly Thr Gly Gly Asn Trp Ile Ala Leu Pro Gln Lys Ile Gly Leu Lys  
 35 40 45  
 Arg Cys Gly Lys Ser Cys Arg Leu Arg Trp Leu Asn Tyr Leu Arg Pro  
 50 55 60  
 Asn Ile Lys His Gly Gly Phe Ser Glu Glu Glu Glu Asn Ile Ile Cys  
 65 70 75 80  
 Ser Leu Tyr Leu Thr Ile Gly Ser Arg Trp Ser Ile Ile Ala Ala Gln  
 85 90 95  
 Leu Pro Gly Arg Thr Asp Asn Asp Ile Lys Asn Tyr Trp Asn Thr Arg  
 100 105 110  
 Leu Lys Lys Lys Leu Ile Asn Lys Gln Arg Lys Glu Leu Gln Glu Ala  
 115 120 125  
 Cys Met Glu Gln Gln Glu Met Met Val Met Met Lys Arg Gln His Gln  
 130 135 140  
 Gln Gln Gln Ile Gln Thr Ser Phe Met Met Arg Gln Asp Gln Thr Met  
 145 150 155 160  
 Phe Thr Trp Pro Leu His His His Asn Val Gln Val Pro Ala Leu Phe  
 165 170 175  
 Arg Ile Lys Pro Thr Arg Phe Ala Thr Lys Lys Met Leu Ser Gln Cys  
 180 185 190  
 Ser Ser Arg Thr Trp Ser Arg Ser Lys Ile Lys Asn Trp Arg Lys Gln  
 195 200 205  
 Thr Ser Ser Ser Ser Arg Phe Asn Asp Asn Ala Phe Asp His Leu Ser  
 210 215 220  
 Phe Ser Gln Leu Leu Leu Asp Pro Asn His Asn His Leu Gly Ser Gly  
 225 230 235 240  
 Glu Gly Phe Ser Met Asn Ser Ile Leu Ser Ala Asn Thr Asn Ser Pro  
 245 250 255  
 Leu Leu Asn Thr Ser Asn Asp Asn Gln Trp Phe Gly Asn Phe Gln Ala  
 Page 35

MBI-0021.txt

260

265

270

Glu Thr Val Asn Leu Phe Ser Gly Ala Ser Thr Ser Thr Ser Ala Asp  
 275 280 285

Gln Ser Thr Ile Ser Trp Glu Asp Ile Ser Ser Leu Val Tyr Ser Asp  
 290 295 300

Ser Lys Gln Phe Phe  
 305

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 Ala Thr Glu Thr Ser Ser Leu Lys Leu Phe Gly Ile Asn Leu Leu Glu  
 5 10 15

acg acg tcg gtt caa aac cag tca tcg gaa cca aga ccc gga tcc gga 152  
 Thr Thr Ser Val Gln Asn Gln Ser Ser Glu Pro Arg Pro Gly Ser Gly  
 20 25 30

tca gga tcc gag tca cgt aag tac gag tgt caa tac tgt tgt aga gag 200  
 Ser Gly Ser Glu Ser Arg Lys Tyr Glu Cys Gln Tyr Cys Cys Arg Glu  
 35 40 45

ttt gct aac tct caa gct ctt ggt ggt cac caa aac gct cac aag aaa 248  
 Phe Ala Asn Ser Gln Ala Leu Gly Gly His Gln Asn Ala His Lys Lys  
 50 55 60 65

gag cgt cag ctt ctt aaa cgt gca cag atg tta gct act cgt ggt ttg 296  
 Glu Arg Gln Leu Leu Lys Arg Ala Gln Met Leu Ala Thr Arg Gly Leu  
 70 75 80

cca cgt cat cat aat ttt cac cct cat acc aat ccg ctt ctc tcc gcc 344  
 Pro Arg His His Asn Phe His Pro His Thr Asn Pro Leu Leu Ser Ala  
 85 90 95

ttc gcg ccg ctg cct cac ctc ctc tct cag ccg cat cct ccg ccg cat 392  
 Phe Ala Pro Leu Pro His Leu Leu Ser Gln Pro His Pro Pro Pro His  
 100 105 110

atg atg ctc tct cct tct tct tcg agt tct aag tgg ctt tac ggt gaa 440  
 Met Met Leu Ser Pro Ser Ser Ser Ser Lys Trp Leu Tyr Gly Glu  
 115 120 125

MBI-0021.txt

cac atg tcg tca caa aac gcc gtt ggg tac ttt cat ggt gga agg gga	488
His Met Ser Ser Gln Asn Ala Val Gly Tyr Phe His Gly Gly Arg Gly	
130 135 140 145	
ctt tac gga ggt ggc atg gag tct atg gcc gga gaa gta aag act cat	536
Leu Tyr Gly Gly Gly Met Glu Ser Met Ala Gly Glu Val Lys Thr His	
150 155 160	
ggg ggt tct ttg ccg gag atg agg agg ttc gcc gga gat agt gat cgg	584
Gly Gly Ser Leu Pro Glu Met Arg Arg Phe Ala Gly Asp Ser Asp Arg	
165 170 175	
agt agc gga att aag tta gag aat ggt att ggg ctg gac ctc cat tta	632
Ser Ser Gly Ile Lys Leu Glu Asn Gly Ile Gly Leu Asp Leu His Leu	
180 185 190	
agc ctt ggg cca tga atgattataa ttttggccca gtaaagatct gtaaaatact	687
Ser Leu Gly Pro	
195	
actaggattt cattttttata gagtattgtt ttttccttaa tttcggttga aattggtgaa	747
tattttttatc tcttacttac caaatctcat atttctatgt atgcgtttgc tttcactttt	807
tttttttata taattcttct tgtaaaaaat gcaatgtgag ttttcttccc tatcattctg	867
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20 25 30	
Gly Ser Gly Ser Glu Ser Arg Lys Tyr Glu Cys Gln Tyr Cys Cys Arg	
35 40 45	
Glu Phe Ala Asn Ser Gln Ala Leu Gly Gly His Gln Asn Ala His Lys	
50 55 60	
Lys Glu Arg Gln Leu Leu Lys Arg Ala Gln Met Leu Ala Thr Arg Gly	
65 70 75 80	
Leu Pro Arg His His Asn Phe His Pro His Thr Asn Pro Leu Leu Ser	
85 90 95	
Ala Phe Ala Pro Leu Pro His Leu Leu Ser Gln Pro His Pro Pro Pro	
100 105 110	

## MBI-0021.txt

His Met Met Leu Ser Pro Ser Ser Ser Ser Lys Trp Leu Tyr Gly  
 115 120 125

Glu His Met Ser Ser Gln Asn Ala Val Gly Tyr Phe His Gly Gly Arg  
 130 135 140

Gly Leu Tyr Gly Gly Gly Met Glu Ser Met Ala Gly Glu Val Lys Thr  
 145 150 155 160

His Gly Gly Ser Leu Pro Glu Met Arg Arg Phe Ala Gly Asp Ser Asp  
 165 170 175

Arg Ser Ser Gly Ile Lys Leu Glu Asn Gly Ile Gly Leu Asp Leu His  
 180 185 190

Leu Ser Leu Gly Pro  
 195

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 1 5 10 15

atg aag caa atc cta cca tca aat gca aag atc tca aaa gaa gca aaa 96  
 Met Lys Gln Ile Leu Pro Ser Asn Ala Lys Ile Ser Lys Glu Ala Lys  
 20 25 30

caa aca gtt caa gaa tgt gca aca gag ttc ata agc ttt gtt aca tgc 144  
 Gln Thr Val Gln Glu Cys Ala Thr Glu Phe Ile Ser Phe Val Thr Cys  
 35 40 45

gaa gca tca gag aag tgc cac agg gag aat cgg aag acg gtg aat gga 192  
 Glu Ala Ser Glu Lys Cys His Arg Glu Asn Arg Lys Thr Val Asn Gly  
 50 55 60

gac gac atc tgg tgg gct ctc agc act ctc ggc ctc gat aac tat gct 240  
 Asp Asp Ile Trp Trp Ala Leu Ser Thr Leu Gly Leu Asp Asn Tyr Ala  
 65 70 75 80

gac gcc gtg ggt agg cat ctt cac aag tac cgt gaa gcc gag aga gaa 288  
 Asp Ala Val Gly Arg His Leu His Lys Tyr Arg Glu Ala Glu Arg Glu  
 85 90 95

aga act gag cac aac aaa ggt agc aat gat agt ggg aat gag aaa gaa 336  
 Arg Thr Glu His Asn Lys Gly Ser Asn Asp Ser Gly Asn Glu Lys Glu



## MBI-0021.txt

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100      105      110
acc aac act aga agt gat gta cag aac caa tcg aca aaa ttt att aga      384
Thr Asn Thr Arg Ser Asp Val Gln Asn Gln Ser Thr Lys Phe Ile Arg
      115      120      125

ggt gtt gag aag gga agc agc tcc tcg gcc cgt tga      420
Val Val Glu Lys Gly Ser Ser Ser Ser Ala Arg
      130      135

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<400> 36

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1      5      10      15

```

```

Met Lys Gln Ile Leu Pro Ser Asn Ala Lys Ile Ser Lys Glu Ala Lys
20      25      30

```

```

Gln Thr Val Gln Glu Cys Ala Thr Glu Phe Ile Ser Phe Val Thr Cys
35      40      45

```

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Glu Ala Ser Glu Lys Cys His Arg Glu Asn Arg Lys Thr Val Asn Gly
50      55      60

```

```

Asp Asp Ile Trp Trp Ala Leu Ser Thr Leu Gly Leu Asp Asn Tyr Ala
65      70      75      80

```

```

Asp Ala Val Gly Arg His Leu His Lys Tyr Arg Glu Ala Glu Arg Glu
85      90      95

```

```

Arg Thr Glu His Asn Lys Gly Ser Asn Asp Ser Gly Asn Glu Lys Glu
100     105     110

```

```

Thr Asn Thr Arg Ser Asp Val Gln Asn Gln Ser Thr Lys Phe Ile Arg
115     120     125

```

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Val Val Glu Lys Gly Ser Ser Ser Ser Ala Arg
130     135

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<210> 37
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<213> Arabidopsis thaliana

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<223> G748

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.MBI-0021.txt

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                                         Met Met Met Glu Thr Arg
                                         1 5

gat cca gct att aag ctt ttc ggt atg aaa atc cct ttt ccg tcg gtt 163
Asp Pro Ala Ile Lys Leu Phe Gly Met Lys Ile Pro Phe Pro Ser Val
                        10 15 20

ttt gaa tcg gca gtt acg gtg gag gat gac gaa gaa gat gac tgg agc 211
Phe Glu Ser Ala Val Thr Val Glu Asp Asp Glu Glu Asp Asp Trp Ser
                        25 30 35

ggc gga gat gac aaa tca cca gag aag gta act cca gag tta tca gat 259
Gly Gly Asp Asp Lys Ser Pro Glu Lys Val Thr Pro Glu Leu Ser Asp
                        40 45 50

aag aac aac aac aac tgt aac gac aac agt ttt aac aat tcg aaa ccc 307
Lys Asn Asn Asn Asn Cys Asn Asp Asn Ser Phe Asn Asn Ser Lys Pro
55 60 65 70

gaa acc ttg gac aaa gag gaa gcg aca tca act gat cag ata gag agt 355
Glu Thr Leu Asp Lys Glu Glu Ala Thr Ser Thr Asp Gln Ile Glu Ser
                        75 80 85

agt gac acg cct gag gat aat cag cag acg aca cct gat ggt aaa acc 403
Ser Asp Thr Pro Glu Asp Asn Gln Gln Thr Thr Pro Asp Gly Lys Thr
                        90 95 100

cta aag aaa ccg act aag att cta ccg tgt ccg aga tgc aaa agc atg 451
Leu Lys Lys Pro Thr Lys Ile Leu Pro Cys Pro Arg Cys Lys Ser Met
                        105 110 115

gag acc aag ttc tgt tat tac aac aac tac aac ata aac cag cct cgt 499
Glu Thr Lys Phe Cys Tyr Tyr Asn Asn Tyr Asn Ile Asn Gln Pro Arg
120 125 130

cat ttc tgc aag gct tgt cag aga tat tgg act gct gga ggg act atg 547
His Phe Cys Lys Ala Cys Gln Arg Tyr Trp Thr Ala Gly Gly Thr Met
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agg aat gtt cct gtg ggg gca gga cgt cgt aag aac aaa agc tca tct 595
Arg Asn Val Pro Val Gly Ala Gly Arg Arg Lys Asn Lys Ser Ser Ser
155 160 165

tct cat tac cgt cac atc act att tcc gag gct ctt gag gct gcg agg 643
Ser His Tyr Arg His Ile Thr Ile Ser Glu Ala Leu Glu Ala Ala Arg
170 175 180

ctt gac ccg ggc tta cag gca aac aca agg gtc ttg agt ttt ggt ctc 691
Leu Asp Pro Gly Leu Gln Ala Asn Thr Arg Val Leu Ser Phe Gly Leu
185 190 195

gaa gct cag cag cag cac gtt gct gct ccc atg aca cct gtt atg aag 739
Glu Ala Gln Gln Gln His Val Ala Ala Pro Met Thr Pro Val Met Lys
200 205 210

cta caa gaa gat caa aag gtc tca aac ggt gct agg aac agg ttt cac 787
Leu Gln Glu Asp Gln Lys Val Ser Asn Gly Ala Arg Asn Arg Phe His
                                         Page 40

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MBI-0021.txt

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Cys Ser Ser Gly	Ser Ser Val Thr Thr	Ser Asn Asn His Ser	Val Asp	
	250	255	260	
gaa tca aga gca	caa agc ggc agt gtt	gaa gca caa atg aac aac		931
Glu Ser Arg Ala	Gln Ser Gly Ser Val	Glu Ala Gln Met Asn Asn		
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aac aac aac aat	aac atg aat ggt tat	gct tgc atc cca ggt	gtt cca	979
Asn Asn Asn Asn	Asn Met Asn Gly Tyr	Ala Cys Ile Pro Gly	Val Pro	
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Trp Pro Tyr Thr	Trp Asn Pro Ala Met	Pro Pro Pro Gly Phe	Tyr Pro	
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Pro Pro Gly Tyr	Pro Met Pro Phe Tyr	Pro Tyr Trp Thr Ile	Pro Met	
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Thr Asn Ser Pro	Thr Leu Gly Lys His	Pro Arg Asp Glu Gly	Ser Ser	
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aaa aag gac aat	gag aca gag cga aaa	cag aag gcc ggg tgc	gtt ctg	1219
Lys Lys Asp Asn	Glu Thr Glu Arg Lys	Gln Lys Ala Gly Cys	Val Leu	
	360	365	370	
gtc ccg aaa acg	ttg aga ata gat gat	cct aac gaa gca gca	aag agc	1267
Val Pro Lys Thr	Leu Arg Ile Asp Asp	Pro Asn Glu Ala Ala	Lys Ser	
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Ser Ile Trp Thr	Thr Leu Gly Ile Lys	Asn Glu Ala Met Cys	Lys Ala	
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ggg ggt atg ttc	aaa ggg ttt gat cat	aag aca aag atg tat	aac aac	1363
Gly Gly Met Phe	Lys Gly Phe Asp His	Lys Thr Lys Met Tyr	Asn Asn	
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gac aaa gct gag	aac tcc cct gtt ctt	tct gct aac cct gct	gct cta	1411
Asp Lys Ala Glu	Asn Ser Pro Val Leu	Ser Ala Asn Pro Ala	Ala Leu	
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tca aga tca cac	aat ttc cat gaa cag	att tag agttacatat	gtatatgtat	1464
Ser Arg Ser His	Asn Phe His Glu Gln	Ile		
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aactcttttc ttctttctag	tgattgcctt tattccttta	catgttttgg ttctctgtac		1584
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MBI-0021.txt

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 1707  
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 35 40 45

Thr Pro Glu Leu Ser Asp Lys Asn Asn Asn Asn Cys Asn Asp Asn Ser  
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Thr Asp Gln Ile Glu Ser Ser Asp Thr Pro Glu Asp Asn Gln Gln Thr  
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Thr Pro Asp Gly Lys Thr Leu Lys Lys Pro Thr Lys Ile Leu Pro Cys  
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Pro Arg Cys Lys Ser Met Glu Thr Lys Phe Cys Tyr Tyr Asn Asn Tyr  
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Lys Asn Lys Ser Ser Ser Ser His Tyr Arg His Ile Thr Ile Ser Glu  
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Ala Leu Glu Ala Ala Arg Leu Asp Pro Gly Leu Gln Ala Asn Thr Arg  
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Val Leu Ser Phe Gly Leu Glu Ala Gln Gln Gln His Val Ala Ala Pro  
 195 200 205

MBI-0021.txt

Met Thr Pro Val Met Lys Leu Gln Glu Asp Gln Lys Val Ser Asn Gly  
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Ala Arg Asn Arg Phe His Gly Leu Ala Asp Gln Arg Leu Val Ala Arg  
 225 230 235 240

Val Glu Asn Gly Asp Asp Cys Ser Ser Gly Ser Ser Val Thr Thr Ser  
 245 250 255

Asn Asn His Ser Val Asp Glu Ser Arg Ala Gln Ser Gly Ser Val Val  
 260 265 270

Glu Ala Gln Met Asn Asn Asn Asn Asn Asn Met Asn Gly Tyr Ala  
 275 280 285

Cys Ile Pro Gly Val Pro Trp Pro Tyr Thr Trp Asn Pro Ala Met Pro  
 290 295 300

Pro Pro Gly Phe Tyr Pro Pro Pro Gly Tyr Pro Met Pro Phe Tyr Pro  
 305 310 315 320

Tyr Trp Thr Ile Pro Met Leu Pro Pro His Gln Ser Ser Ser Pro Ile  
 325 330 335

Ser Gln Lys Cys Ser Asn Thr Asn Ser Pro Thr Leu Gly Lys His Pro  
 340 345 350

Arg Asp Glu Gly Ser Ser Lys Lys Asp Asn Glu Thr Glu Arg Lys Gln  
 355 360 365

Lys Ala Gly Cys Val Leu Val Pro Lys Thr Leu Arg Ile Asp Asp Pro  
 370 375 380

Asn Glu Ala Ala Lys Ser Ser Ile Trp Thr Thr Leu Gly Ile Lys Asn  
 385 390 395 400

Glu Ala Met Cys Lys Ala Gly Gly Met Phe Lys Gly Phe Asp His Lys  
 405 410 415

Thr Lys Met Tyr Asn Asn Asp Lys Ala Glu Asn Ser Pro Val Leu Ser  
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## MBI-0021.txt

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 atg ggt ggt cgt aaa cca tgt tgt gat gag gtt gga tta aga aag ggt 227  
 Met Gly Gly Arg Lys Pro Cys Cys Asp Glu Val Gly Leu Arg Lys Gly  
 1 5 10 15  
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 Pro Trp Thr Val Glu Glu Asp Gly Lys Leu Val Asp Phe Leu Arg Ala  
 20 25 30  
 cgt ggc aac tgc ggt ggt ggt gga gga gga tgg tgc tgg aga gac gtg 323  
 Arg Gly Asn Cys Gly Gly Gly Gly Gly Gly Trp Cys Trp Arg Asp Val  
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 Pro Lys Leu Ala Gly Leu Arg Arg Cys Gly Lys Ser Cys Arg Leu Arg  
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 Trp Thr Asn Tyr Leu Arg Pro Asp Leu Lys Arg Gly Leu Phe Thr Glu  
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 Glu Glu Ile Gln Leu Val Ile Asp Leu His Ala Arg Leu Gly Asn Arg  
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 Ile Asp Pro Asn Thr His Arg Arg Phe Asp Gln Gln Lys Val Asn Glu  
 130 135 140  
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 Glu Glu Thr Ile Leu Val Asn Asp Pro Lys Pro Leu Ser Glu Thr Glu  
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MBI-0021.txt

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Thr Met Leu Leu Ser Gly Asp Ile Thr Ser Ser Cys Ser Ser Ser Ser
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      225                      230                      235                      240

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Gly Cys Phe Asp Val
      245

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35                      40                      45

Pro Lys Leu Ala Gly Leu Arg Arg Cys Gly Lys Ser Cys Arg Leu Arg
50                      55                      60

Trp Thr Asn Tyr Leu Arg Pro Asp Leu Lys Arg Gly Leu Phe Thr Glu
65                      70                      75                      80

Glu Glu Ile Gln Leu Val Ile Asp Leu His Ala Arg Leu Gly Asn Arg
85                      90                      95

Trp Ser Lys Ile Ala Val Glu Leu Pro Gly Arg Thr Asp Asn Asp Ile
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Lys Asn Tyr Trp Asn Thr His Ile Lys Arg Lys Leu Ile Arg Met Gly
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MBI-0021.txt

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Val Ser Val Ala Leu Lys Asn Asp Thr Ser Ala Val Leu Ser Gly Asn  
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Leu Asn Gln Leu Ala Asp Val Asp Gly Asp Asp Gln Pro Trp Ser Phe  
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Leu Met Glu Asn Asp Glu Gly Gly Gly Gly Asp Ala Ala Gly Glu Leu  
 195 200 205

Thr Met Leu Leu Ser Gly Asp Ile Thr Ser Ser Cys Ser Ser Ser Ser  
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 Phe Asp Thr Gln Lys Gly Phe Gly Phe Ile Thr Pro Asp Asp Gly Gly  
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gac gat ctc ttc gtt cac cag tcc tcc atc aga tct gag ggt ttc cgt 202  
 Asp Asp Leu Phe Val His Gln Ser Ser Ile Arg Ser Glu Gly Phe Arg  
 35 40 45

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 Ser Leu Ala Ala Glu Glu Ala Val Glu Phe Glu Val Glu Ile Asp Asn  
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MBI-0021.txt

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Glu Gly Gly Gly Gly Tyr Gly Gly Gly	Gly Gly Gly Tyr Gly Gly Gly			
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Gly Gly Gly Gly Gly Gly Ser Cys Tyr	Ser Cys Gly Glu Ser Gly His			
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MBI-0021.txt

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 50 55 60

Asn Asn Asn Arg Pro Lys Ala Ile Asp Val Ser Gly Pro Asp Gly Ala  
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Pro Val Gln Gly Asn Ser Gly Gly Gly Ser Ser Gly Gly Arg Gly Gly  
 85 90 95

Phe Gly Gly Gly Arg Gly Gly Gly Arg Gly Ser Gly Gly Gly Tyr Gly  
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 115 120 125

Ser Asp Cys Tyr Lys Cys Gly Glu Pro Gly His Met Ala Arg Asp Cys  
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 Met Gly Asn Ser Ser Glu Glu Pro Lys Pro Pro Thr

MBI-0021.txt

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		15					20					25																																				
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## MBI-0021.txt

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 Glu Glu Leu Ala Arg Lys Val Glu Ala Leu Thr Ala Glu Asn Met Ala  
 285 290 295  
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 Leu Arg Ser Glu Leu Asn Gln Leu Asn Glu Lys Ser Asp Lys Leu Arg  
 305 310 315  
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 Lys Phe His Gln Leu Leu Asp Thr Lys Pro Arg Ala Lys Ala Val Ala  
 365 370 375 380  
 gca ggc tga atcgatggta attcatgtcg atttctactt aatttgtcga 1325  
 Ala Gly  
 cataaacaac gaaaataagt gctactaatt tcagaaaaac ttgatagata gatagtatag 1385  
 tagagagaga gagagagaga gaggtgtgat gattattgat ctataaattt tcggagagag 1445  
 agagggagaa agagaaactt ttcctccaga tgaaaatttg gtgttatggg ttgttactgt 1505  
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 1 5 10 15  
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 20 25 30  
 Trp Ala Ala Met Gln Ala Tyr Tyr Gly Pro Arg Val Ala Met Pro Pro  
 35 40 45

MBI-0021.txt

Tyr Tyr Asn Ser Ala Met Ala Ala Ser Gly His Pro Pro Pro Tyr  
 50 55 60

Met Trp Asn Pro Gln His Met Met Ser Pro Ser Gly Ala Pro Tyr Ala  
 65 70 75 80

Ala Val Tyr Pro His Gly Gly Gly Val Tyr Ala His Pro Gly Ile Pro  
 85 90 95

Met Gly Ser Leu Pro Gln Gly Gln Lys Asp Pro Pro Leu Thr Thr Pro  
 100 105 110

Gly Thr Leu Leu Ser Ile Asp Thr Pro Thr Lys Ser Thr Gly Asn Thr  
 115 120 125

Asp Asn Gly Leu Met Lys Lys Leu Lys Glu Phe Asp Gly Leu Ala Met  
 130 135 140

Ser Leu Gly Asn Gly Asn Pro Glu Asn Gly Ala Asp Glu His Lys Arg  
 145 150 155 160

Ser Arg Asn Ser Ser Glu Thr Asp Gly Ser Thr Asp Gly Ser Asp Gly  
 165 170 175

Asn Thr Thr Gly Ala Asp Glu Pro Lys Leu Lys Arg Ser Arg Glu Gly  
 180 185 190

Thr Pro Thr Lys Asp Gly Lys Gln Leu Val Gln Ala Ser Ser Phe His  
 195 200 205

Ser Val Ser Pro Ser Ser Gly Asp Thr Gly Val Lys Leu Ile Gln Gly  
 210 215 220

Ser Gly Ala Ile Leu Ser Pro Gly Val Ser Ala Asn Ser Asn Pro Phe  
 225 230 235 240

Met Ser Gln Ser Leu Ala Met Val Pro Pro Glu Thr Trp Leu Gln Asn  
 245 250 255

Glu Arg Glu Leu Lys Arg Glu Arg Arg Lys Gln Ser Asn Arg Glu Ser  
 260 265 270

Ala Arg Arg Ser Arg Leu Arg Lys Gln Ala Glu Thr Glu Glu Leu Ala  
 275 280 285

Arg Lys Val Glu Ala Leu Thr Ala Glu Asn Met Ala Leu Arg Ser Glu  
 290 295 300

MBI-0021.txt

Leu Asn Gln Leu Asn Glu Lys Ser Asp Lys Leu Arg Gly Ala Asn Ala  
305 310 315 320

Thr Leu Leu Asp Lys Leu Lys Cys Ser Glu Pro Glu Lys Arg Val Pro  
325 330 335

Ala Asn Met Leu Ser Arg Val Lys Asn Ser Gly Ala Gly Asp Lys Asn  
340 345 350

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355 360 365

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gca ttt aac act cga aca ata aaa aat gaa gaa gag aca cac ccg ccg 96  
Ala Phe Asn Thr Arg Thr Ile Lys Asn Glu Glu Glu Thr His Pro Pro  
20 25 30  
  
gag caa gaa gcc aca ata gcc gtt aga tca tca tca tca tcg gat ctg 144  
Glu Gln Glu Ala Thr Ile Ala Val Arg Ser Ser Ser Ser Ser Asp Leu  
35 40 45  
  
acg gcc gag aag cgt ccg gat aag atc ata gca tgt cca aga tgc aag 192  
Thr Ala Glu Lys Arg Pro Asp Lys Ile Ile Ala Cys Pro Arg Cys Lys  
50 55 60  
  
agc atg gag aca aag ttc tgt tac ttc aac aac tac aac ggt aat cag 240  
Ser Met Glu Thr Lys Phe Cys Tyr Phe Asn Asn Tyr Asn Gly Asn Gln  
65 70 75 80  
  
cct cga cac ttt tgt aaa ggc tgc cac cgt tac tgg acc gcc ggt ggt 288  
Pro Arg His Phe Cys Lys Gly Cys His Arg Tyr Trp Thr Ala Gly Gly  
85 90 95  
  
gca ctc cgg aac gtt ccc gtc ggc gcc ggt cgt cgg aag tcc aaa cca 336  
Ala Leu Arg Asn Val Pro Val Gly Ala Gly Arg Arg Lys Ser Lys Pro  
100 105 110  
  
cct ggt cgt gtc gtg gtt ggt atg ctt gga gat gga aat ggt gtt cgc 384  
Pro Gly Arg Val Val Val Gly Met Leu Gly Asp Gly Asn Gly Val Arg

MBI-0021.txt

115	120	125	
caa gtc gag ctt ata aat ggc ttg ctc gtt gag gag tgg cag cat gcc			432
Gln Val Glu Leu Ile Asn Gly Leu Leu Val Glu Glu Trp Gln His Ala			
130	135	140	
gca gcc gca gct cac ggt agt ttc cgg cat gat ttt ccc atg aag cgg			480
Ala Ala Ala Ala His Gly Ser Phe Arg His Asp Phe Pro Met Lys Arg			
145	150	155	160
ctc cgg tgt tac tcc gac ggt caa tcg tgc tga			513
Leu Arg Cys Tyr Ser Asp Gly Gln Ser Cys			
	165	170	

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<400> 46

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20	30
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35	45
Thr Ala Glu Lys Arg Pro Asp Lys Ile Ile Ala Cys Pro Arg Cys Lys	
50	60
Ser Met Glu Thr Lys Phe Cys Tyr Phe Asn Asn Tyr Asn Gly Asn Gln	
65	80
Pro Arg His Phe Cys Lys Gly Cys His Arg Tyr Trp Thr Ala Gly Gly	
85	95
Ala Leu Arg Asn Val Pro Val Gly Ala Gly Arg Arg Lys Ser Lys Pro	
100	110
Pro Gly Arg Val Val Val Gly Met Leu Gly Asp Gly Asn Gly Val Arg	
115	125
Gln Val Glu Leu Ile Asn Gly Leu Leu Val Glu Glu Trp Gln His Ala	
130	140
Ala Ala Ala Ala His Gly Ser Phe Arg His Asp Phe Pro Met Lys Arg	
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Leu Arg Cys Tyr Ser Asp Gly Gln Ser Cys	

MBI-0021.txt  
170

165

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Met Glu Leu Asn Arg Ser Glu Ala Asp Glu Ala Lys Ala Glu Thr Thr 15  
1 5 10 15  
ccc acc ggt gga gcc acc agc tca gcc aca gcc tct ggc tct tcc tcc 157  
Pro Thr Gly Gly Ala Thr Ser Ser Ala Thr Ala Ser Gly Ser Ser Ser 30  
20 25  
gga cgt cgt cca cgt ggt cgt cct gca ggt tcc aaa aac aaa ccc aaa 205  
Gly Arg Arg Pro Arg Gly Arg Pro Ala Gly Ser Lys Asn Lys Pro Lys 45  
35 40  
cct ccg acg att ata act aga gat agt cct aac gtc ctt aga tca cac 253  
Pro Pro Thr Ile Ile Thr Arg Asp Ser Pro Asn Val Leu Arg Ser His 60  
50 55  
gtt ctt gaa gtc acc tcc ggt tgc gac ata tcc gag gca gtc tcc acc 301  
Val Leu Glu Val Thr Ser Gly Ser Asp Ile Ser Glu Ala Val Ser Thr 80  
65 70 75  
tac gcc act cgt cgc ggc tgc ggc gtt tgc att ata agc ggc acg ggt 349  
Tyr Ala Thr Arg Arg Gly Cys Gly Val Cys Ile Ile Ser Gly Thr Gly 95  
85 90  
gcg gtc act aac gtc acg ata cgg caa cct gcg gct ccg gct ggt gga 397  
Ala Val Thr Asn Val Thr Ile Arg Gln Pro Ala Ala Pro Ala Gly Gly 110  
100 105 110  
ggt gtg att acc ctg cat ggt cgg ttt gac att ttg tct ttg acc ggt 445  
Gly Val Ile Thr Leu His Gly Arg Phe Asp Ile Leu Ser Leu Thr Gly 125  
115 120  
act gcg ctt cca ccg cct gca cca ccg gga gca gga ggt ttg acg gtg 493  
Thr Ala Leu Pro Pro Pro Ala Pro Pro Gly Ala Gly Gly Leu Thr Val 140  
130 135  
tat cta gcc gga ggt caa gga caa gtt gta gga ggg aat gtg gct ggt 541  
Tyr Leu Ala Gly Gly Gln Gly Gln Val Val Gly Gly Asn Val Ala Gly 160  
145 150 155  
tcg tta att gct tcg gga ccg gta gtg ttg atg gct gct tct ttt gca 589  
Ser Leu Ile Ala Ser Gly Pro Val Val Leu Met Ala Ala Ser Phe Ala 175  
165 170  
aac gca gtt tat gat agg tta ccg att gaa gag gaa gaa acc cca ccg 637  
Asn Ala Val Tyr Asp Arg Leu Pro Ile Glu Glu Glu Glu Thr Pro Pro 175



## MBI-0021.txt

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180          185          190
ccg aga acc acc ggg gtg cag cag cag cag ccg gag gcg tct cag tcg      685
Pro Arg Thr Thr Gly Val Gln Gln Gln Gln Pro Glu Ala Ser Gln Ser
195          200          205

tcg gag gtt acg ggg agt ggg gcc cag gcg tgt gag tca aac ctc caa      733
Ser Glu Val Thr Gly Ser Gly Ala Gln Ala Cys Glu Ser Asn Leu Gln
210          215          220

ggg gga aat ggt gga gga ggt gtt gct ttc tac aat ctt gga atg aat      781
Gly Gly Asn Gly Gly Gly Gly Val Ala Phe Tyr Asn Leu Gly Met Asn
225          230          235          240

atg aac aat ttt caa ttc tcc ggg gga gat att tac ggt atg agc ggc      829
Met Asn Asn Phe Gln Phe Ser Gly Gly Asp Ile Tyr Gly Met Ser Gly
245          250          255

ggg agc gga gga ggt ggt ggc ggt gcg act aga ccc gcg ttt tag      874
Gly Ser Gly Gly Gly Gly Gly Gly Ala Thr Arg Pro Ala Phe
260          265          270

agtttttagcg ttttggtgac accttttggt gcgtttgcgt gtttgacctc aaactactag      934

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20          25          30

Gly Arg Arg Pro Arg Gly Arg Pro Ala Gly Ser Lys Asn Lys Pro Lys
35          40          45

Pro Pro Thr Ile Ile Thr Arg Asp Ser Pro Asn Val Leu Arg Ser His
50          55          60

Val Leu Glu Val Thr Ser Gly Ser Asp Ile Ser Glu Ala Val Ser Thr
65          70          75          80

Tyr Ala Thr Arg Arg Gly Cys Gly Val Cys Ile Ile Ser Gly Thr Gly
85          90          95

Ala Val Thr Asn Val Thr Ile Arg Gln Pro Ala Ala Pro Ala Gly Gly
100          105          110

Gly Val Ile Thr Leu His Gly Arg Phe Asp Ile Leu Ser Leu Thr Gly

```

MBI-0021.txt

115

120

125

Thr Ala Leu Pro Pro Pro Ala Pro Pro Gly Ala Gly Gly Leu Thr Val  
 130 135 140

Tyr Leu Ala Gly Gly Gln Gly Gln Val Val Gly Gly Asn Val Ala Gly  
 145 150 155 160

Ser Leu Ile Ala Ser Gly Pro Val Val Leu Met Ala Ala Ser Phe Ala  
 165 170 175

Asn Ala Val Tyr Asp Arg Leu Pro Ile Glu Glu Glu Glu Thr Pro Pro  
 180 185 190

Pro Arg Thr Thr Gly Val Gln Gln Gln Gln Pro Glu Ala Ser Gln Ser  
 195 200 205

Ser Glu Val Thr Gly Ser Gly Ala Gln Ala Cys Glu Ser Asn Leu Gln  
 210 215 220

Gly Gly Asn Gly Gly Gly Gly Val Ala Phe Tyr Asn Leu Gly Met Asn  
 225 230 235 240

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 245 250 255

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 260 265 270

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gga gaa gac gcc ggc ggc ggc gat gaa tat agg att ccg gaa tgg gaa 97  
 Gly Glu Asp Ala Gly Gly Gly Asp Glu Tyr Arg Ile Pro Glu Trp Glu  
 15 20 25 30

att ggt tta ccc aac gga gat gat ttg act ccg tta tct caa tat cta 145  
 Ile Gly Leu Pro Asn Gly Asp Asp Leu Thr Pro Leu Ser Gln Tyr Leu  
 35 40 45

MBI-0021.txt

gtc ccg tcg att ctc gcg tta gct ttc agc atg atc cca gaa cga agc	193
Val Pro Ser Ile Leu Ala Leu Ala Phe Ser Met Ile Pro Glu Arg Ser	
50 55 60	
cgt aca att cac gac gtc aat cgc gcg tcg caa atc acg ctc tct tcg	241
Arg Thr Ile His Asp Val Asn Arg Ala Ser Gln Ile Thr Leu Ser Ser	
65 70 75	
ttg aga agc agt acc aat gct tcg tct gtg atg gag gag gtc gtg gat	289
Leu Arg Ser Ser Thr Asn Ala Ser Ser Val Met Glu Glu Val Val Asp	
80 85 90	
cga gtt gaa tcg agt gtt cca gga tca gat ccg aag aaa cag aag aaa	337
Arg Val Glu Ser Ser Val Pro Gly Ser Asp Pro Lys Lys Gln Lys Lys	
95 100 105 110	
tcg gat ggt ggt gaa gca gcg gcg gtg gag gat tcc acg gcg gag gaa	385
Ser Asp Gly Gly Glu Ala Ala Val Glu Asp Ser Thr Ala Glu Glu	
115 120 125	
gga gac tcc ggg cct gaa gac gcg tct ggg aag aca tcg aaa cga ccg	433
Gly Asp Ser Gly Pro Glu Asp Ala Ser Gly Lys Thr Ser Lys Arg Pro	
130 135 140	
cgt tta gtg tgg aca ccg cag cta cac aag aga ttt gtg gac gtt gtg	481
Arg Leu Val Trp Thr Pro Gln Leu His Lys Arg Phe Val Asp Val Val	
145 150 155	
gct cat cta ggg att aaa aac gca gtg ccg aag acg att atg cag ctg	529
Ala His Leu Gly Ile Lys Asn Ala Val Pro Lys Thr Ile Met Gln Leu	
160 165 170	
atg aac gtg gaa gga ctt act cgt gag aac gtt gcg tct cat ttg cag	577
Met Asn Val Glu Gly Leu Thr Arg Glu Asn Val Ala Ser His Leu Gln	
175 180 185 190	
aaa tat agg ctt tac ctt aaa cgg att caa gga ttg acg acg gaa gaa	625
Lys Tyr Arg Leu Tyr Leu Lys Arg Ile Gln Gly Leu Thr Thr Glu Glu	
195 200 205	
gat cct tat tcg tcg tcg gat cag ctc ttc tct tca acg ccg gtt cct	673
Asp Pro Tyr Ser Ser Ser Asp Gln Leu Phe Ser Ser Thr Pro Val Pro	
210 215 220	
cca cag agc ttt caa gac ggc gga gga agt aac gga aag ttg ggg gtt	721
Pro Gln Ser Phe Gln Asp Gly Gly Gly Ser Asn Gly Lys Leu Gly Val	
225 230 235	
ccg gtt ccg gtt ccg tcg atg gtg cct att cca ggc tat ggg aat caa	769
Pro Val Pro Val Pro Ser Met Val Pro Ile Pro Gly Tyr Gly Asn Gln	
240 245 250	
atg ggt atg caa gga tat tat caa cag tat agt aac cat ggc aat gaa	817
Met Gly Met Gln Gly Tyr Tyr Gln Gln Tyr Ser Asn His Gly Asn Glu	
255 260 265 270	
tca aac caa tat atg atg cag cag aat aag ttt gga aca atg gtg aca	865
Ser Asn Gln Tyr Met Met Gln Gln Asn Lys Phe Gly Thr Met Val Thr	
275 280 285	
tat cct tct gtt ggt ggt ggt gac gtg aat gac aag taa atggatctta	914
Tyr Pro Ser Val Gly Gly Gly Asp Val Asn Asp Lys	
290 295	

MBI-0021.txt

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aaggctata atttgctcta cagagagata ctgggtcttg gcttatgggt tattttccca 974
cttcatgagg ttgttgtagac ttttaattct ccatgttttc cacacaagtc tttattgcct 1034
ttgtatagaa aatgatttcg agaaaatcac tgggaagctt ggtattgttg gaggatgaag 1094
ccttctatga atgatttagt ttcctactgt ctccattctt tatgaggtaa taaagccttc 1154
ttttgctcat cgctttagt cttcttaaat tcaagacagc gtcacatgtt tggtcggtta 1214
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Asp Ala Gly Gly Gly Asp Glu Tyr Arg Ile Pro Glu Trp Glu Ile Gly
20          25          30

```

```

Leu Pro Asn Gly Asp Asp Leu Thr Pro Leu Ser Gln Tyr Leu Val Pro
35          40          45

```

```

Ser Ile Leu Ala Leu Ala Phe Ser Met Ile Pro Glu Arg Ser Arg Thr
50          55          60

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```

Ile His Asp Val Asn Arg Ala Ser Gln Ile Thr Leu Ser Ser Leu Arg
65          70          75          80

```

```

Ser Ser Thr Asn Ala Ser Ser Val Met Glu Glu Val Val Asp Arg Val
85          90          95

```

```

Glu Ser Ser Val Pro Gly Ser Asp Pro Lys Lys Gln Lys Lys Ser Asp
100          105          110

```

```

Gly Gly Glu Ala Ala Ala Val Glu Asp Ser Thr Ala Glu Glu Gly Asp
115          120          125

```

```

Ser Gly Pro Glu Asp Ala Ser Gly Lys Thr Ser Lys Arg Pro Arg Leu
130          135          140

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```

Val Trp Thr Pro Gln Leu His Lys Arg Phe Val Asp Val Val Ala His
145          150          155          160

```

MBI-0021.txt

Leu Gly Ile Lys Asn Ala Val Pro Lys Thr Ile Met Gln Leu Met Asn  
165 170 175

Val Glu Gly Leu Thr Arg Glu Asn Val Ala Ser His Leu Gln Lys Tyr  
180 185 190

Arg Leu Tyr Leu Lys Arg Ile Gln Gly Leu Thr Thr Glu Glu Asp Pro  
195 200 205

Tyr Ser Ser Ser Asp Gln Leu Phe Ser Ser Thr Pro Val Pro Pro Gln  
210 215 220

Ser Phe Gln Asp Gly Gly Gly Ser Asn Gly Lys Leu Gly Val Pro Val  
225 230 235 240

Pro Val Pro Ser Met Val Pro Ile Pro Gly Tyr Gly Asn Gln Met Gly  
245 250 255

Met Gln Gly Tyr Tyr Gln Gln Tyr Ser Asn His Gly Asn Glu Ser Asn  
260 265 270

Gln Tyr Met Met Gln Gln Asn Lys Phe Gly Thr Met Val Thr Tyr Pro  
275 280 285

Ser Val Gly Gly Gly Asp Val Asn Asp Lys  
290 295

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Asn Phe Leu Val Pro Phe Glu Glu Thr Asn Val Leu Thr Phe Phe Ser  
5 10 15

tct tct tct tcc tct tct ctt tct tct cct tct ttc ccc att cac aac 152  
Ser Ser Ser Ser Ser Ser Leu Ser Ser Pro Ser Phe Pro Ile His Asn  
20 25 30

tct tcc tcc act act act act cat gca cct cta ggg ttt tct aat aat 200  
Ser Ser Ser Thr Thr Thr Thr His Ala Pro Leu Gly Phe Ser Asn Asn  
35 40 45

## MBI-0021.txt

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ctt cag ggt gga gga ccc ttg gga tca aag gtg gtt aat gat gat cag      248
Leu Gln Gly Gly Gly Pro Leu Gly Ser Lys Val Val Asn Asp Asp Gln
50                      55                      60                      65

gag aat ttt gga ggt gga act aac aat gat gct cat tct aat tct tgg      296
Glu Asn Phe Gly Gly Gly Thr Asn Asn Asp Ala His Ser Asn Ser Trp
70                      75                      80

tgg aga tca aat agt gga agt gga gat atg aag aac aaa gtg aag ata      344
Trp Arg Ser Asn Ser Gly Ser Gly Asp Met Lys Asn Lys Val Lys Ile
85                      90                      95

agg agg aaa cta aga gag cca aga ttc tgt ttc caa acc aaa agc gat      392
Arg Arg Lys Leu Arg Glu Pro Arg Phe Cys Phe Gln Thr Lys Ser Asp
100                     105                     110

gtt gat gtt ctt gac gat ggc tac aaa tgg cgt aaa tat ggt cag aaa      440
Val Asp Val Leu Asp Asp Gly Tyr Lys Trp Arg Lys Tyr Gly Gln Lys
115                     120                     125

gtc gtc aag aac agc ctt cac ccc agg agt tat tac aga tgc aca cac      488
Val Val Lys Asn Ser Leu His Pro Arg Ser Tyr Tyr Arg Cys Thr His
130                     135                     140                     145

aac aac tgt agg gtg aaa aag aga gtg gag cga cta tcg gaa gat tgt      536
Asn Asn Cys Arg Val Lys Lys Arg Val Glu Arg Leu Ser Glu Asp Cys
150                     155                     160

aga atg gtg att act act tac gaa ggt cgt cac aac cac att ccc tct      584
Arg Met Val Ile Thr Thr Tyr Glu Gly Arg His Asn His Ile Pro Ser
165                     170                     175

gat gac tcc act tct cct gac cat gat tgt ctc tct tcc ttt taa      629
Asp Asp Ser Thr Ser Pro Asp His Asp Cys Leu Ser Ser Phe
180                     185                     190

catctctttc tatatatcta tatatagaca gttatatgtg cacatataga tgtgtgatat      689
attgcatatt tgatattgca tgtgtttttc aagagtatgt catcagatgt tatgcatata      749
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20 25 30

Asn Ser Ser Ser Thr Thr Thr Thr His Ala Pro Leu Gly Phe Ser Asn  
35 40 45

MBI-0021.txt

Asn Leu Gln Gly Gly Gly Pro Leu Gly Ser Lys Val Val Asn Asp Asp  
50 55 60

Gln Glu Asn Phe Gly Gly Gly Thr Asn Asn Asp Ala His Ser Asn Ser  
65 70 75 80

Trp Trp Arg Ser Asn Ser Gly Ser Gly Asp Met Lys Asn Lys Val Lys  
85 90 95

Ile Arg Arg Lys Leu Arg Glu Pro Arg Phe Cys Phe Gln Thr Lys Ser  
100 105 110

Asp Val Asp Val Leu Asp Asp Gly Tyr Lys Trp Arg Lys Tyr Gly Gln  
115 120 125

Lys Val Val Lys Asn Ser Leu His Pro Arg Ser Tyr Tyr Arg Cys Thr  
130 135 140

His Asn Asn Cys Arg Val Lys Lys Arg Val Glu Arg Leu Ser Glu Asp  
145 150 155 160

Cys Arg Met Val Ile Thr Thr Tyr Glu Gly Arg His Asn His Ile Pro  
165 170 175

Ser Asp Asp Ser Thr Ser Pro Asp His Asp Cys Leu Ser Ser Phe  
180 185 190

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<223> G592

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atg gat tca aat aat cat ctc tac gac ccg aat ccc acc ggg tcg ggt 168  
Met Asp Ser Asn Asn His Leu Tyr Asp Pro Asn Pro Thr Gly Ser Gly  
1 5 10 15  
ctt ctt cgt ttt aga tca gct ccg agc tct gtt ctc gcc gct ttt gtt 216  
Leu Leu Arg Phe Arg Ser Ala Pro Ser Ser Val Leu Ala Ala Phe Val  
20 25 30  
gac gac gac aag att ggt ttc gac tcc gat agg ttg ctt tca aga ttc 264  
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## MBI-0021.txt

Asp Asp Asp Lys Ile Gly Phe Asp Ser Asp Arg Leu Leu Ser Arg Phe  
 35 40 45  
 gtg acc tct aat ggc gtt aac gga gat ctg ggt tca cct aaa ttc gag 312  
 Val Thr Ser Asn Gly Val Asn Gly Asp Leu Gly Ser Pro Lys Phe Glu  
 50 55 60  
 gat aag tct ccg gtt tct tta acg aac acc tct gtt tca tac gcc gcc 360  
 Asp Lys Ser Pro Val Ser Leu Thr Asn Thr Val Ser Tyr Ala Ala  
 65 70 75 80  
 act ctg ccg cca ccg ccg cag ctt gag ccg tct agt ttt ctg ggt ttg 408  
 Thr Leu Pro Pro Pro Gln Leu Glu Pro Ser Ser Phe Leu Gly Leu  
 85 90 95  
 ccg ccg cat tac ccg agg cag agt aaa ggg ata atg aac tct gtt ggt 456  
 Pro Pro His Tyr Pro Arg Gln Ser Lys Gly Ile Met Asn Ser Val Gly  
 100 105 110  
 ttg gat cag ttt ctc ggt atc aat aat cat cac acc aaa cca gtt gaa 504  
 Leu Asp Gln Phe Leu Gly Ile Asn Asn His His Thr Lys Pro Val Glu  
 115 120 125  
 tct aat ctt ctc cgt caa agc agc tct cca gcc gga atg ttt act aat 552  
 Ser Asn Leu Leu Arg Gln Ser Ser Ser Pro Ala Gly Met Phe Thr Asn  
 130 135 140  
 ctc tct gac caa aac ggt tat ggt tca atg agg aat ttg atg aat tac 600  
 Leu Ser Asp Gln Asn Gly Tyr Gly Ser Met Asn Leu Met Asn Tyr  
 145 150 155 160  
 gaa gaa gat gaa gag agt cca tct aat tcc aat gga tta aga cgc cat 648  
 Glu Glu Asp Glu Glu Ser Pro Ser Asn Ser Asn Gly Leu Arg Arg His  
 165 170 175  
 tgc agt ctc tct tca agg cca cct tct tca ctt gga atg ctt tct caa 696  
 Cys Ser Leu Ser Ser Arg Pro Pro Ser Ser Leu Gly Met Leu Ser Gln  
 180 185 190  
 ata cct gaa atc gca ccc gaa act aat ttt cca tat agc cat tgg aat 744  
 Ile Pro Glu Ile Ala Pro Glu Thr Asn Phe Pro Tyr Ser His Trp Asn  
 195 200 205  
 gat cca tcc agc ttt att gat aac tta tcc tca ctt aaa aga gaa gcc 792  
 Asp Pro Ser Ser Phe Ile Asp Asn Leu Ser Ser Leu Lys Arg Glu Ala  
 210 215 220  
 gag gac gat gga aaa ttg ttt ctc gga gct cag aac gga gag tcc ggg 840  
 Glu Asp Asp Gly Lys Leu Phe Leu Gly Ala Gln Asn Gly Glu Ser Gly  
 225 230 235 240  
 aat cgt atg cag tta ctg tct cat cat ttg agc cta cca aag tca tca 888  
 Asn Arg Met Gln Leu Leu Ser His His Leu Ser Leu Pro Lys Ser Ser  
 245 250 255  
 tct aca gcc tct gac atg gtt tca gtg gat aag tat ctt cag cta caa 936  
 Ser Thr Ala Ser Asp Met Val Ser Val Asp Lys Tyr Leu Gln Leu Gln  
 260 265 270  
 gat tct gtt cct tgt aaa atc aga gcc aaa cgt ggt tgc gct aca cat 984  
 Asp Ser Val Pro Cys Lys Ile Arg Ala Lys Arg Gly Cys Ala Thr His  
 275 280 285



## MBI-0021.txt

cct cga agc atc gct gaa cgg gta aga aga acg cgg ata agc gag cga 1032  
 Pro Arg Ser Ile Ala Glu Arg Val Arg Arg Thr Arg Ile Ser Glu Arg  
 290 295 300

atg agg aag tta caa gag ctt gtt cct aac atg gac aag caa acc aac 1080  
 Met Arg Lys Leu Gln Glu Leu Val Pro Asn Met Asp Lys Gln Thr Asn  
 305 310 315 320

act tcg gat atg ttg gat tta gct gtg gat tac atc aaa gat tta caa 1128  
 Thr Ser Asp Met Leu Asp Leu Ala Val Asp Tyr Ile Lys Asp Leu Gln  
 325 330 335

aga cag tat aag att tta aac gac aac aga gct aac tgt aag tgt atg 1176  
 Arg Gln Tyr Lys Ile Leu Asn Asp Asn Arg Ala Asn Cys Lys Cys Met  
 340 345 350

aac aag gag aag aag tca ata tag ggcgcaacaa agtgtgtagt agataggact 1230  
 Asn Lys Glu Lys Lys Ser Ile  
 355

aaaaagcagg gagaaggaca agaaagaaac aatgtcatgt ctgaatattt tttagccgaa 1290

acagacccaaa ttgtctatgt aagctctcga gaaaagcatc tgcttccaac aaaattctaa 1350

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aaa 1413

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Asp Asp Asp Lys Ile Gly Phe Asp Ser Asp Arg Leu Leu Ser Arg Phe  
 35 40 45

Val Thr Ser Asn Gly Val Asn Gly Asp Leu Gly Ser Pro Lys Phe Glu  
 50 55 60

Asp Lys Ser Pro Val Ser Leu Thr Asn Thr Ser Val Ser Tyr Ala Ala  
 65 70 75 80

Thr Leu Pro Pro Pro Gln Leu Glu Pro Ser Ser Phe Leu Gly Leu  
 85 90 95

Pro Pro His Tyr Pro Arg Gln Ser Lys Gly Ile Met Asn Ser Val Gly  
 100 105 110

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Leu Asp Gln Phe Leu Gly Ile Asn Asn His His Thr Lys Pro Val Glu  
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Ser Asn Leu Leu Arg Gln Ser Ser Ser Pro Ala Gly Met Phe Thr Asn  
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Leu Ser Asp Gln Asn Gly Tyr Gly Ser Met Arg Asn Leu Met Asn Tyr  
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Glu Glu Asp Glu Glu Ser Pro Ser Asn Ser Asn Gly Leu Arg Arg His  
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Cys Ser Leu Ser Ser Arg Pro Pro Ser Ser Leu Gly Met Leu Ser Gln  
 180 185 190

Ile Pro Glu Ile Ala Pro Glu Thr Asn Phe Pro Tyr Ser His Trp Asn  
 195 200 205

Asp Pro Ser Ser Phe Ile Asp Asn Leu Ser Ser Leu Lys Arg Glu Ala  
 210 215 220

Glu Asp Asp Gly Lys Leu Phe Leu Gly Ala Gln Asn Gly Glu Ser Gly  
 225 230 235 240

Asn Arg Met Gln Leu Leu Ser His His Leu Ser Leu Pro Lys Ser Ser  
 245 250 255

Ser Thr Ala Ser Asp Met Val Ser Val Asp Lys Tyr Leu Gln Leu Gln  
 260 265 270

Asp Ser Val Pro Cys Lys Ile Arg Ala Lys Arg Gly Cys Ala Thr His  
 275 280 285

Pro Arg Ser Ile Ala Glu Arg Val Arg Arg Thr Arg Ile Ser Glu Arg  
 290 295 300

Met Arg Lys Leu Gln Glu Leu Val Pro Asn Met Asp Lys Gln Thr Asn  
 305 310 315 320

Thr Ser Asp Met Leu Asp Leu Ala Val Asp Tyr Ile Lys Asp Leu Gln  
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 Phe Gly Ser Trp Ser Gly Arg Ile Val Gly Val Gly Ser Ser Ala Asp  
 205 210 215 220  
 tct aaa ccg tgg tgc gac ccg gtg atg gag gcg cgt ttg tca ctg ttg  
 Ser Lys Pro Trp Cys Asp Pro Val Met Glu Ala Arg Leu Ser Leu Leu  
 225 230 235 722  
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 Trp Thr Lys Glu Glu Asp Gln Arg Leu Val Asp Tyr Ile Arg Asn His  
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 Gly Glu Gly Cys Trp Arg Ser Leu Pro Lys Ser Ala Gly Leu Leu Arg  
 35 40 45  
 Cys Gly Lys Ser Cys Arg Leu Arg Trp Ile Asn Tyr Leu Arg Pro Asp  
 50 55 60  
 Leu Lys Arg Gly Asn Phe Thr Asp Asp Glu Asp Gln Ile Ile Ile Lys  
 65 70 75 80  
 Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu Ile Ala Gly Arg Leu  
 85 90 95  
 Pro Gly Arg Thr Asp Asn Glu Ile Lys Asn Tyr Trp Asn Thr His Ile  
 100 105 110  
 Lys Arg Lys Leu Leu Ser His Gly Ile Asp Pro Gln Thr His Arg Gln  
 115 120 125  
 Ile Asn Glu Ser Lys Thr Val Ser Ser Gln Val Val Val Pro Ile Gln  
 130 135 140  
 Asn Asp Ala Val Glu Tyr Ser Phe Ser Asn Leu Ala Val Lys Pro Lys  
 145 150 155 160  
 Thr Glu Asn Ser Ser Asp Asn Gly Ala Ser Thr Ser Gly Thr Thr Thr  
 165 170 175

MBI-0021.txt

Asp Glu Asp Leu Arg Gln Asn Gly Glu Cys Tyr Tyr Ser Asp Asn Ser  
 180 185 190

Gly His Ile Lys Leu Asn Leu Asp Leu Thr Leu Gly Phe Gly Ser Trp  
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Ser Gly Arg Ile Val Gly Val Gly Ser Ser Ala Asp Ser Lys Pro Trp  
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Cys Asp Pro Val Met Glu Ala Arg Leu Ser Leu Leu  
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MBI-0021.txt

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